UNIVERSITAT POLITÈCNICA DE CATALUNYA BARCELONATECH

Durability of resistance to Meloidogyne spp. mediated by R-genes in solanaceous and cucurbitaceous crops

Helio Adán García Mendívil

Universitat Politècnica de Catalunya
Departament d'Enginyeria Agroalimentària i Biotecnologia
Programa de Doctorat Tecnologia Agroalimentària i Biotecnologia

Durability of resistance to *Meloidogyne* spp. mediated by *R*-genes in solanaceous and cucurbitaceous crops

PhD dissertation

Helio Adán García Mendívil

PhD Supervisor: **Dr. Francisco Javier Sorribas Royo**

Castelldefels, España September 16, 2019

The work presented in this thesis has been developed in the Integrated Plant-Parasitic Nematode Research Group of the Department of Agri-Food Engineering and Biotechnology of the UPC (Universitat Politècnica de Catalunya-BarcelonaTech, in the framework of the research projects:

"Efecto de la resistencia de genes *R* y la inducida por hongos endofitos en la epidemiología de *Meloidogyne* y la producción y calidad de la cosecha en solanaceas-cucurbitaceas". Funded by Ministerio de Economća y Competitividad (AGL2013-49040-C2-1R).

"Estrategias de gestión de germoplasma vegetal resistente a *Meloidogyne* para evitar la selección de virulencia". Funded by Ministerio de Economća y Competitividad (AGL2017-89785-R).

And with help of the pre-doctoral grant provided by the Consejo Nacional de Ciencía y Tecnología (No. 411638).

Acknowledgments

Thanks are given to Consejo Nacional de Ciencía y Tecnología for financing the pre-doctoral grant that allowed the work done in this thesis.

To all people from UPC that were involved in the realization of this thesis. Specially to my PhD supervisor, Dr. Francisco Javier Sorribas, for the opportunity to do this work, his invaluable teachings and the immense patience towards me.

To the lab 20 team, Alex, Sergi, Isma, Ana, Ary, Nuria, Aida, Miqel and Roger for their friendship and time invested to make possible this work. I could not imagine better lab partners. To Sheila and María from UPC for the patience in the lab and their help in the field. To Miqel and Dani from Agropólis for their help and agronomic advises. To Montserrat Marine for being the nicest administrative I ever met.

To Andrea and Carlos for becoming a second family, and for making me feel like at home since the first day I arrived to Spain. To Anna, Tomas and Hector for their friendship that stopped me from going crazy in this journey.

Those who past away since I left México. Jairo and David, I hope you are skating together. Grandpa, your obstinate personality still lives here.

To my parents for having the confidence to allowed me to explore and made my own decisions and mistakes, the freedom to become who I wanted to be, and that second chance when I needed. To my sisters for giving me more nephews to enjoy since I started this work. And to Venecia, Obiel and Amelie, I'm sorry not being there for you all this time.

Contents

Durability of resistance to *Meloidogyne* spp. mediated by *R*-genes in solanaceous and cucurbitaceous crops

Helio Adán García Mendívil

Abstract

Watermelon, *Citrullus lanatus* var. *lanatus*, and eggplant, *Solanum melongena*, are two major crops commonly grafted in order to overcome soilborne diseases. However, there are currently no commercially available rootstocks resistant to root-knot nematodes (RKN), *Meloidogyne* spp., infection in the *Cucurbitacea* family, and also a narrow diversity in the *Solanaceae*, mostly in tomato and pepper. In order find alternatives to address this problem, the main objective of this thesis was to determine the durability of resistance to *Meloidogyne* of *Citrullus amarus* and *Solanum torvum* as potential rootstocks for watermelon and eggplant, respectively. In the first part of this document, the work conducted with the cucurbits is presented in chapters one and two, while chapters three and four correspond to work done with solanaceous.

Durabilty of *Citrullus amarus* resistance to *Meloidogyne*: the response of two *Citrullus amarus* accessions, BGV0005164 and BGV0005167, was assessed against different *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* isolates in pot experiments and against *M. incognita* in plastic greenhouse. (i) In the pot experiments, plants were inoculated with a second-stage juvenile per cm^3 of sterile sand and maintained in a growth chamber at 25 °C for 50 days. The watermelon cv. Sugar Baby was included as a susceptible control for comparison. At the end of the experiments, the number of egg masses and eggs per plant was determined, and the reproduction index was calculated as the percentage of the number of eggs produced in the *C. amarus* accessions with regard to that produced in the susceptible cv. Sugar Baby. (ii) In the plastic greenhouse experiment, the ungrafted watermelon cv. Sugar Baby and watermelon grafted onto each of the *C. amarus* accessions and onto the watermelon rootstock cv. Robusta were cultivated from May to August 2016 in plots with nematode densities from 46 to 1392 J2 per 250 cm^3 of soil at transplantation. At the end of the experiment, the galling index and the number of eggs per plant were determined, and the reproduction index was calculated. (iii) Additionally, the compatibility of the two accessions with the watermelon cv. Sugar Baby and the effect on fruit quality (weight, size, shape, firmness, pH, total soluble solids, and flesh color) were assessed under a hydroponic system in a greenhouse. The commercial rootstocks cv. Cobalt and cv. Robusta were also included. Moreover (iv) The response of ungrafted and grafted watermelon cv. Sugar baby onto the *C. amarus* accessions BGV0005164 and BGV0005167 submitted to increasing densities of *M. incognita* and *M. javanica* was studied in pot experiments to determine the maximum multiplication rate, the maximum population density and the equilibrium density of the root-knot nematode species and the effect on shoot dry biomass of watermelon. (v) In plastic greenhouse conditions, the ungrafted and grafted watermelon onto both *C. amarus* accessions, and onto the *C. lanatus* rootstock cv. Robusta were cultivated for two consecutive years in the same plots to assess the level of resistance to *M. incognita* and crop yield. (vi) Additionally, after the second crop, the putative selection for virulence in the nematode subpopulation originated in the ungrafted and grafted watermelon was assessed in pot experiments. The results showed that (i) all the *Meloidogyne* isolates produced fewer egg masses and eggs per plant in the accessions than in Sugar Baby. Both accessions performed as resistant against *M. arenaria*, and from highly to moderately resistant to *M. incognita* and *M. javanica* in pot experiments. (ii) In the plastic greenhouse experiment, both *C. amarus* accessions performed as resistant to *M. incognita*. (iii) Both *C. amarus* accessions were compatible with the watermelon cv. Sugar Baby, but only the BGV0005167 accession did not influence the fruit quality. (iv) The maximum multiplication rate, the maximum population density

and the equilibrium density values of both *Meloidogyne* species were lower in grafted than ungrafted watermelon. (v) In the plastic greenhouse experiment, the nematode densities in soil at transplantation ranged from 1 to 53 J2 per 100 cm^3 of soil in 2017 and did not differ between grafted and ungrafted watermelons. At the end of the crop, the galling index and the number of eggs per plant was higher in ungrafted than in grafted watermelon both years. The *C. amarus* accessions performed from highly resistant to resistant to *M. incognita*, and the rootstock cv. Robusta from moderately resistant in 2017 to slightly resistant in 2018. All grafted watermelons yielded more kg per plant than the ungrafted in both years. (vi) The repeated cultivation of grafted watermelon onto *C. amarus* accessions did not select for virulence. In conclusion, the BGV0005167 accession is a promising rootstock for managing the three tropical root-knot nematode species without influencing watermelon fruit quality. The results of this study highlight the poorer host status of CI64 and CI67 accessions to *M. incognita* and *M. javanica* compared to watermelon; the stability of the *C. amarus* resistance; and the beneficial effect of *C. amarus* on watermelon yield when cultivated in *Meloidogyne* infested soils.

Durabilty of Solanum torvum resistance to Meloidogyne: several experiments were carried out to assess the performance of commercial *Solanum torvum* cultivars against *Meloidogyne incognita* and *M. javanica* isolates from Spain. (i) The response of *S. torvum* rootstock cultivars Brutus, Espina, Salutamu and Torpedo against *M. incognita* and *Mi1.2* (a)virulent *M. javanica* isolates was determined in pot experiments, and of cv. Brutus to an *N*-virulent isolate of *M. incognita*, compared with that of the eggplant cv. Cristal. (ii) The relationship between the initial and final population densities of *M. javanica* on ungrafted and grafted 'Cristal' onto the *S. torvum* 'Brutus' was assessed, together with the effect on dry shoot biomass. (iii) Finally, the population growth rate and the resistance level of the four *S. torvum* cultivars against *M. incognita* was assessed under plastic greenhouse conditions in two cropping seasons. (iv) The eggplant *Solanum melongena* cv. Cristal, either ungrafted or grafted onto the *S. torvum* rootstock cv. Brutus was cultivated for two consecutive years in the same plots in a plastic greenhouse to assess the level of resistance to *M. incognita* and crop yield. (v) At the end of the second crop, the putative selection for virulence of the nematode subpopulations coming from infected ungrafted and grafted eggplant was assessed in the eggplant and in *S. torvum* in a pot experiment. The results showed that: (i) all *S. torvum* rootstocks responded as resistant to the *M. incognita* isolates and from highly resistant to susceptible against *M. javanica* isolates. (ii) The maximum multiplication rate of *M. javanica* on the ungrafted or grafted eggplant were 270 and 49, respectively, and the equilibrium density were 1318 and 2056 eggs and J2 per 100 cm^3 soil, respectively. The tolerance of the ungrafted eggplant was 10.9 J2 per 100 cm^3 soil, and the minimum relative dry shoot biomass was 0.76. (iii) The population growth rate of *M. incognita* on eggplant cv. Cristal differed from that of the *S. torvum* cultivars in both cropping seasons. (iv) Nematode population densities at transplantation in 2017 ranged from 2 to 378 J2 per 100 cm^3 of soil and did not differ between ungrafted and grafted eggplant. At the end of each crop, higher galling index and number of nematodes in soil and in roots were registered in ungrafted than grafted eggplant. The grafted eggplant performed as resistant in 2017 and as highly resistant in 2018. Eggplant yield did not differ irrespective of grafting in 2017 after being cultivated for 135 days, but it differed after 251 days of cultivation in 2018. (v) In the pot experiment, *S. torvum* performed as resistant to both *M. incognita* subpopulations. However, the *M. incognita* subpopulation obtained from roots of *S. torvum* produced 49.4% less egg masses and 56% less eggs per plant in the eggplant than the nematode subpopulation obtained from roots of the eggplant cv. Cristal. These results suggest that *S. torvum* is a valuable rootstock for managing the two *Meloidogyne* species irrespective of the (a)virulence status, and revealed that the infective and reproductive fitness of the nematode decrease without having been selected for virulence.

General introduction

Durability of resistance to *Meloidogyne* spp. mediated by *R*-genes in solanaceous and cucurbitaceous crops

Helio A. García-Mendívil

PhD Dissertation

Meloidogyne spp.

Biology

Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging plant parasitic nematodes worldwide. This genus comprises more than 100 species, however, most of the crop yield losses are caused by four of them: the tropical species *M. arenaria*, *M. incognita* and *M. javanica*, and the temperate species *M. hapla* (Jones et al. 2013). The widened global warming can favor the expansion and proliferation of the RKN tropical species in areas where the temperate RKN species predominates. This fact evidence the importance of focusing research efforts for designing management strategies to the tropical RKN species.

RKN are obligated parasites that require a suitable host plant for life cycle completion (Fig. 1). The infective preparasitic vermiform second-stage juveniles (J2) of *Meloidogyne* moves through the soil and penetrate the root behind the tip, by using their protractible stylet and releasing secretions containing cell-wall-degrading enzymes (Abad et al. 2003). J2 then migrate intercellularly between the cortical cells towards the root tip where they make a U-turn to later enter into the vascular cylinder and moves until stablishing a feeding site. Each J2 is able to induce the redifferentiation of five to seven parenchymatic root cells into multinucleate and hypertrophied feeding cells, the so-called giant cells, from which get the nutrients needed for its life cycle completion (Nyczepir and Thomas 2009). The accumulation of these giant cells is responsible for the characteristic galled tissue present in infected root systems, and for the disturbance of plant development, defenses, and metabolism (Shukla et al. 2018). Once infection occurs, juveniles become sedentary and assume a "sausage" shape as increases its size. J2 then moults

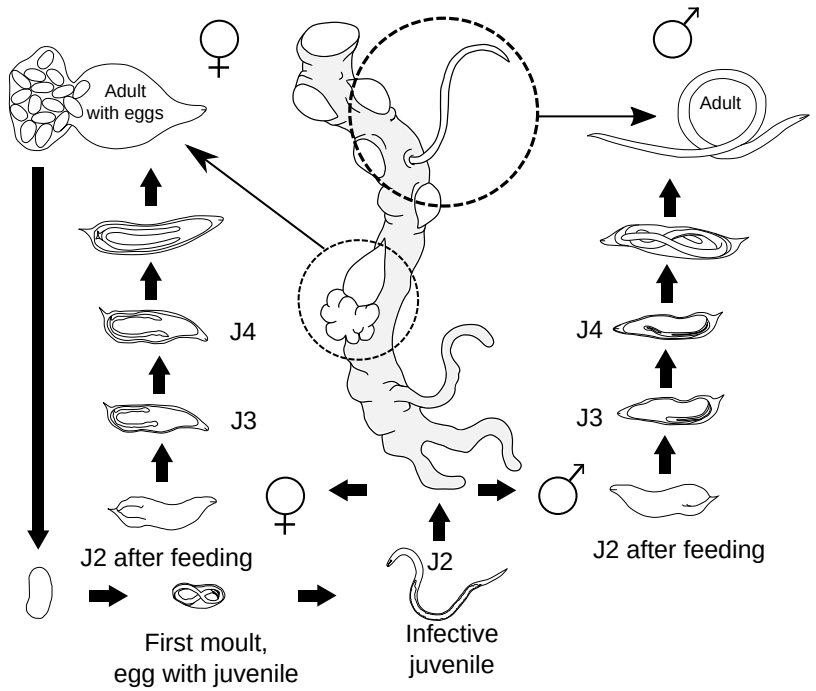


Figure 1: Diagram of the life cycle of the root-knot nematode, *Meloidogyne*. J2: second-stage juvenile; J3: third-stage juvenile; J4: fourth-stage juvenile (adapted from Moens et al. 2009)

three times achieving the adult stage. The tropical species, *M. arenaria, M. incognita* and *M. javanica*, reproduce parthenogenetically. The sedentary pearl-shaped adult female keeps feeding from the giant cells and is able to lay c. 300-500 eggs inside a gelatinous mass. Under unfavourable conditions (high nematode density, scarcity of food or stressed plants) the juveniles develop into males. Interestingly, some studies have found to increase stimulation towards maleness by cropping several cucurbit species (Fassuliotis 1970; Walters et al. 2006; Expósito et al. 2019).

Meloidogyne spp. is a poikilothermic organism, meaning that temperature influence its life cycle and determine its length (Tyler 1933). The nematode development occurs between 10 and 32 °C, and needs to accumulate an amount of degree-days (K) at a certain basal temperature (Tb) to complete its life cycle (Trudgill 1995). For instance, it has been reported that *Meloidogyne* spp. requires between 600 and 700 accumulated degree-days with a base temperature of 10 °C to complete its life cycle in tomato (Ferris 1985). However, the thermal time requirements for life cycle completion can differ between RKN populations of a given species depending of its geographic origin, showing its ability to adapt to environmental factors, i.e., optimal temperatures of 25-30°C and 32-34°C were found in populations of *M. javanica* from Australia and California, respectively (Ferris and Van Gundy 1979). Such adaptive ability may be one of the main reasons of its success in spreading globally, and also evidence its importance for modern agriculture.

Economic importance

The occurrence of soilborne diseases and pests have increased in recent years as consequence of the intensive cultivation needed to supply a growing population in limited land resources (Thies et al. 2015b). A comprehensive summary of the estimation of yield losses caused by RKN in several crops have been published by Greco and Di Vito (2009). The cucurbit and solanaceous crops are two of the crop families most frequently cultivated worldwide that are severely affected by RKN. Regarding cucurbit crops, maximum yield losses of 88, 53 and 35% in cucumber, zucchini and watermelon, respectively, have been estimated under greenhouse conditions (Giné et al. 2014; Vela et al. 2014; López-Gómez et al. 2014, 2015). RKN are also one of the most damaging soilborne pathogens for solanaceous crops, specially under protected cultivation, with maximum yield losses of 94, 95 and 100% reported for pepper, eggplant and tomato, respectively (Giné et al. 2017; Hallmann and Meressa 2018).

Population dynamics and yield losses

Population dynamics studies the factors that determine the temporal oscillation of densities of individuals from the same specie living in a certain area. The nematode population density at sowing or transplanting of a crop is related with its productivity. The proper modeling of population dynamics allows to estimate the densities variability over time in relation with influencing factors, and therefore, to relate them with the yield losses that nematodes could cause. Modeling the damage levels enable to calculate parameters such the tolerance limit (T), the maximum population density above which yield losses start to occur, and the maximum yield losses (m). These parameters, along with the maximum reproduction rate (a)(Pf/Pi at low densities; Ferris 1985), and the equilibrium density, that initial population at which nematode receive just enough supply of nutrients to maintain the population density at the same level from begin at the end of the growing season; permits to evaluate the importance of a determined nematode from a growing area, or the effect of management strategies. These quantitative studies constitute the basis for populations' evolution understanding of specific patosystems with typical agro-environmental conditions from determine growing area, and to design and implement effective and durable management strategies (Sorriba and Verdejo-Lucas 2011; Fig 2).

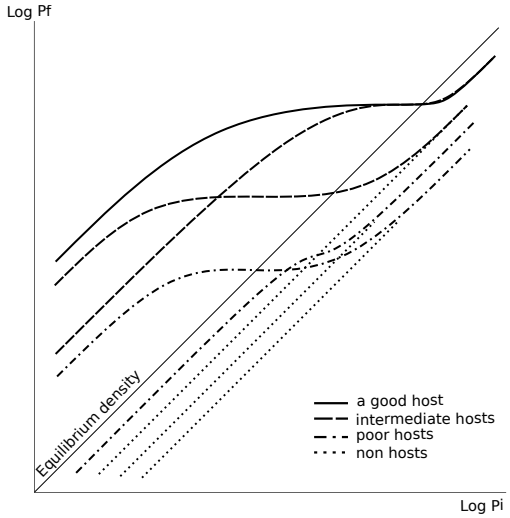


Figure 2: Relation between initial and final densities in experiments with a nematode on a good host, intermediate hosts, poor hosts, and a nonhost. Pi and Pf: initial and final densities on logarithmic scales. Equilibrium density: $Pf = Pi$ (adapted from Seinhorst 1970).

Population density fluctuations are affected by denso-independent and denso-dependent factors. Denso-independent factors, such climate, environmental conditions, and intraspecific competition, influence from outside of population, while denso-dependent include interspecific competition and the action of antagonist or predators. Two phases can be distinguish: one during the host plant cultivation in which nematode have enough nutrients to increase its population density, and the second during either no cropping periods in which there is no food supply and population density do not increase, or decrease depending on the nematode and the duration of periods with no host cultivation.

The multiplication growth rate (Pf/Pi), that is, the relationship between the nematode population density at transplanting (Pi) and at the end of the crop (Pf) is consider a good indicator of population growth. In absence of competition between individuals for limiting resources, the Pf/Pi maximize (a), and the relationship with Pi its a straight line ($Pf = aPi$; thus $Pf/Pi = a$). However, increases in Pi induce competition between individuals for healthy plant tissue, can induce alteration in sex differentiation or to reduce in female fecundity, thus Pf/Pi decreases. In the case of nematodes that reproduce parthenogenetically, such *Meloidogyne* spp., the relationship between Pf/Pi and Pi follows an inverse potential function ($Pf/Pi = aPi^{-b}$, where a is the maximum multiplication rate, and $-b$ is the decrease rate of the population as Pi increase). The maximum multiplication rate (a) is a good indicator of the plant host status to Meloidogyne. Higher values of a indicate that the plant is a good host and low values, a pooor host (Ferris 1985).

The population growth rate can also be useful to estimate the Pi at which $Pf/Pi = 1$, the equilibrium density (E). This can be calculated from the regression equation obtained from linearizing the relationship between the Pi and the multiplication rate (Pf/Pi). The population growth rate is also a useful indicator that allows to compare between plant species and/or germplasms, as well as the effectiveness of control methods (Talavera et al. 2009; Giné and Sorribas 2017; Expósito et al. 2018; Fig 3).

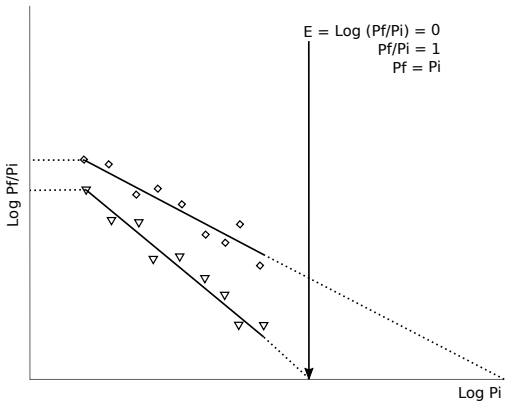


Figure 3: Relation between initial population density (Pi) and multiplication rate (Pf/Pi) of RKN on two hypothetical germplasms (adapted from Ferris 1985).

Regarding to crop yield losses, there are two parameters that can be calculated by estimating the relationship between increasing levels of Pi and the relative crop biomass or yield according to the Seinhort's damage model (Seinhorst 1998):

$$\begin{aligned} y &= m + (1-m)0.95^{\frac{Pi}{T-1}} && for Pi > T \\ y &= 1 && for Pi \leq T \\ y &= m && for Pi \longrightarrow \infty \end{aligned}$$

The nematode population density above which yield loss start to occur is defined as the nematode damage threshold level or the tolerance limit of the crop (T). This value, along with the the minimum relative yield (m) are important parameters to characterize the response of a crop plant to a nematode population. The parameter T manifests itself at small nematode densities and m at larger ones (Greco and Di Vito 2009; Fig 4).

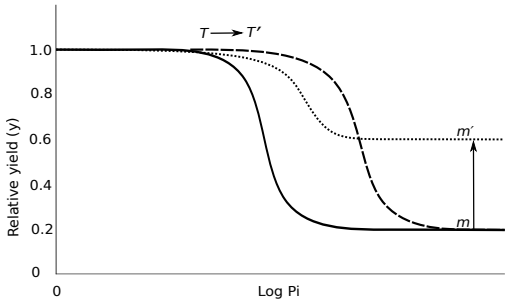


Figure 4: Relationship between relative yield and the initial nematode population density of three hypothetical germplasms with different values of T and m (adapted from Schomaker and Been 2013).

Plant resistance

The most commonly used strategy for RKN management has been, until recent years, the use of fumigant and non fumigant nematicides (Nyczepir and Thomas 2009). However, regulations such the European directive 2009/128/EC and the U.S. Clean Air Act (US Environmental Protection Agency 2012), have brought special attention on the research and development of environmentally friendlier management strategies. A promising alternative strategy involves the use of resistant plants, those whose have the ability to suppress infection development, and/or reproduction of plant-parasitic nematodes (Roberts 2009). Plant resistance has proven to be effective and economically profitable (Sorribas et al. 2005), and also reduce yield losses of the follwing crop (Ornat et al. 1997; Talavera et al. 2009; Westphal 2011; Thies et al. 2015a; Giné and Sorribas 2017; Expósito et al. 2019).

The resistance levels of a determined plant germplasm can be categorized in relation to a susceptible standard (Hadisoeganda 1982). Expressed as the percentage of RKN reproduction compared to that in a susceptible standard, this parameter is called the reproduction index (RI; Fig 5).

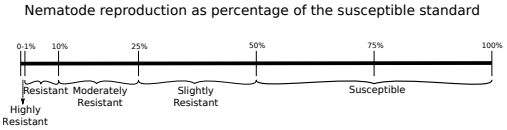


Figure 5: A diagrammatic representation of the continuum of susceptibility and resistance to nematode reproduction within a crop germplasm pool (adapted from Starr and Mercer 2009).

Despite its advantages, the expression of plant resistance can be affected by several factors, such the genetic background of the plant cultivar and/or the nematode species or population (Cortada et al. 2008). The selection of virulent nematode populations due to repeated cultivation of the same resistance gene (Verdejo-Lucas et al. 2009; Giné and Sorribas 2017; Expósito et al. 2019). Moreover, there are only a few resistance genes introgressed into commercial solanaceous cultivars at present, and none in cucurbits. That are, the *Me1, Me3* and *N* gene in pepper, and the *Mi1.2* gene in tomato. Soil temeprature can also affect the expression of plant resistance. It is known that the *Mi1.2* gene is affected at soil temperatures above 30 °C, while the resistance of the *N* gene is reported to be partially lost at 32 °C (Araujo et al. 1982; Thies and Fery 1998). Thus,plant resistance is a valuable management tool but it should be used in a proper manner to maximize its effectiveness. Moreover, more research are needed to identify new resistance sources for increasing the *R*-genes that could be used in rotation schemes .

Grafting vegetables onto resistant rootstocks is an effective method to control soilborne pathogens when no commercial cultivars are available (Miguel et al. 2004; Cohen et al. 2007; Thies et al. 2016; Kumar et al. 2018). This control method was essentially rediscovered in the past two decades and quickly expanded to become a common practice at the present (Thies et al. 2015b;

Kyriacou et al. 2017). Grafting has expanded mainly in the Cucurbitaceae and the Solanaceae families (Moncada et al. 2013). The use of anatomical and physiological compatible graft combinations improve the plant performance under biotic or abiotic stress conditions when compared with that of the ungrafted scion, allowing rapid response to new pathogen races without the prolonged screening and selection required for breeding resistance into cultivars (Davis et al. 2008). Although the tolerance and resistance to abiotic and biotic stresses, respectively, along with an increasing productivity have been the main drivers of rootstock selection and breeding, the effect of grafting on fruit quality is also another important factor to take into account (Kyriacou et al. 2017).

Some strategies proposed to avoid the selection for RKN virulence or to reduce the level of virulence and crop yield losses consider to include resistant and susceptible crops in rotation sequences (Talavera et al. 2009), the use of crops of two different resistant plant species (Expósito et al. 2019), or to pyramid different *R*-genes (Djian-Caporalino et al. 2014). However, as literature reviewed points out, most of the research done have been focusing on tomato and pepper. Thus, the aim of this thesis was to increase the diversity of resistance sources against RKN by potentially effective rootstocks for two species of the most economically important botanical families cultivated, the cucurbitaseous watermelon (*Citrullus lanatus* var. *lanatus*), and the solanaseous eggplant (*Solanum melongena*). A brief description of these two crops and their potential rootstocks is presented in the next two sections.

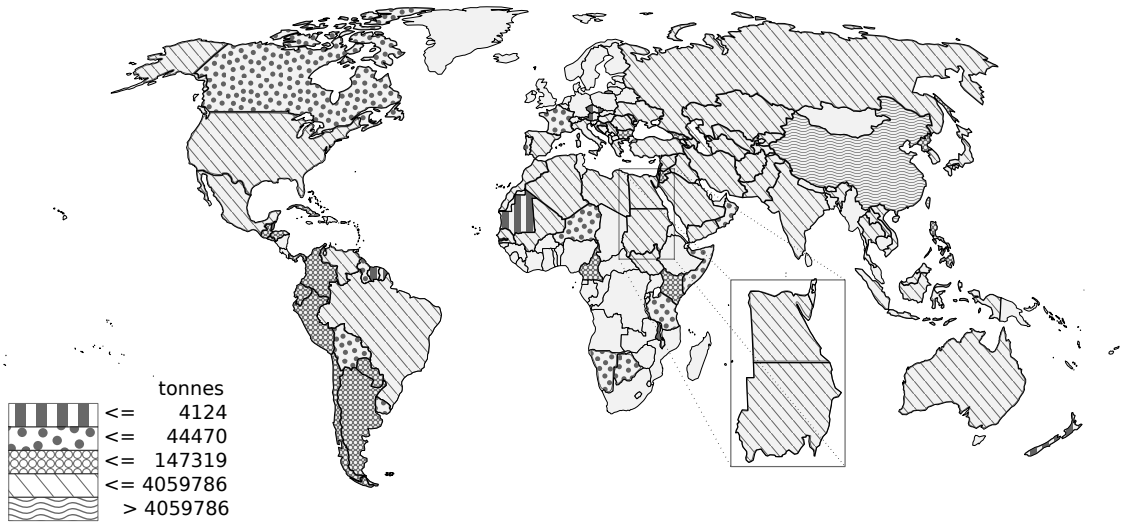


Figure 6: Origin and production quantities of watermelons in 2017 by country (FAOSTAT, 2019; Renner et al. 2019)

Watermelon

Importance

Watermelon is the most cultivated cucurbit crop worldwide. The global production in 2017 was ca. 118 million t in ca. 3.4 million ha, 38% of the arable land destined to cultivate for this botanical family, with an estimated value of $48.9B (FAOSTAT 2017; Fig 6). In spite of its origin theorized in the African continent, most of the watermelon are produced in Asia, with 67% of total world production in China. Within the European Union (EU), Spain was the main watermelon producer during 2017, with 20,026 ha harvested to produce 1.1 million t of fruit marketed with an estimated value of $331M, 68% of which were exported (MAPA 2019).

The watermelon fruit composition is 93% water, with small amounts of protein, fat, minerals, and vitamins. The major nutritional fruit components are carbohydrates (6.4 g/100 g), vitamin A (590 IU), and lycopene (4,100 μg/100g, range 2300–7200), an anticarcinogenic compound found in red flesh watermelon, even in higher amounts than in tomato, pink grapefruit, or guava (Wehner 2008).

Origin

Watermelon is a diploid creeping monoecious crop that belongs to the *Cucurbitaceae* family. The taxonomy of the genus *Citrullus* have had major misapplication of names until recent years, including that of watermelon, *C. lanatus*, itself (Chomicki and Renner 2015; Renner et al. 2014). The Southern African region is reported to be the center of diversity and probably the center of origin of most of the *Citrullus* species (Dane and Lang 2004; Rubatsky 2001; Robinson and Decker-Walters 1997). However, four hypotheses have been proposed for the origin of watermelon: i) that it descends from the northern African colocynth (*C. colocynthis*, Singh 1978; Sain et al. 2002; McCreight et al. 2013). ii) That it derives from the South African citron melon, *C. amarus*, previously referred as *C. lanatus* var. *citroides* (Robinson and Decker-Walters 1997; Maynard and Maynard 2000; Rubatsky 2001; Chomicki and Renner 2015). iii) That it stems from the West African *C. mucosospermus* (Guo et al. 2013; Chomicki and Renner 2015), and iv) Recent research with polygenomic analyses of nuclear gene sequences suggest that the white-fleshed Sudanese Kordophan melon (*Citrullus vulgaris*) is the closest relative of domesticated watermelon that was originated and domesticated in north-eastern Africa (Renner et al. 2019; Fig 6).

Cultivation challenges

The modern cultivated watermelon has a narrow genetic base (Szamosi et al. 2009; Solmaz et al. 2010; Nimmakayala et al. 2014) attributed to many years of domestication and selection of its desirable horticultural traits such as red-scarlet flesh color and sweetness (Levi et al. 2001b; Hwang et al. 2011; Lambel et al. 2014; Nimmakayala et al. 2014). The continued use of cultivars with narrow genetic base for breeding resulted in reduced gene diversity among American watermelon accessions (Wang et al. 2015; Zhang et al. 2016). This has derived in susceptibility of crops to random and emerging biotic and abiotic stresses (Levi et al. 2001a; Mo et al. 2016). In spite of being consider a less suitable host for RKN than other cucurbit crops, watermelon maximum yield losses of 37% have been recently reported in Spain (López-Gómez et al. 2014). Moreover, as the next section will better describe, modern watermelons are commonly grafted onto commercial rootstocks owing its resistance to fusarium, however, these rootstocks are not resistant to *Meloidogyne* (Kokalis-Burelle and Rosskopf 2011; López-Gómez et al. 2016; Giné et al. 2017).

Grafting

A detailed history of cucurbit grafting have been done by Davis (2008). The primary motive for grafting cucurbits is to avoid damage caused by soilborne pests and pathogens when genetic or chemical approaches for disease management are not available (Oda 2002). Research on cucurbit grafting began in the 1920s with the use of *Cucurbita moschata* as a rootstock for watermelon, but then bottle gourd (*Lagenaria siceraria*) soon became the preferred rootstock. By the year 1998, approximately 95% of watermelon and oriental melons were grafted onto squash or bottlegourd rootstocks in Japan, Korea, and Taiwan (Oda and Lee 2003). Possibly because of the widespread use of bottle gourd rootstocks, there are reports of plants affected by Fusarium wilt, probably the most common and damaging soilborne disease of cucurbit crops worldwide, caused by *Fusarium oxysporum* Schltdl. Moreover both rootstocks are susceptible to *Meloidogyne* infection (Kokalis-Burelle and Rosskopf 2011; López-Gómez et al. 2016; Giné et al. 2017). Hence, an alter-

native genetic source of resistance to both pathogens must be found and properly characterized in order to be able to design an effective and widely applicable RKN management strategy.

A potential alternative rootstock for watermelon, is its close relative citron or preserving melon, *C. amarus*. The flesh of the citron is white or green, and may vary from bland to bitter tasting. Its rind is used to make pickles, and the fruit are feeding to livestock (Wehner 2008). Conversely to watermelon, citron melon exhibits wider genetic variation (Levi et al. 2001b) suggesting its genetic worthiness as a source of valuable genes for breeding (Ngwepe et al. 2019). This rootstock have show to be resistant to several soil pathogens such as fusarium wilt, gummy stem blight, powdery mildew, potyviruses, and some species and populations of *Meloidogyne* (Gusmini et al. 2005; Huitrón et al. 2007; Guner and Wehner 2008; Tetteh et al. 2010; Thies et al. 2016). In spite of it, there is little information about it use to control RKN in the EU, thus, research on its response to local population must be done before include it in a management strategy.

Eggplant

Importance

Eggplant, *Solanum melongena*, is the third most widely cultivated Solanaceous fruiting crop after potato and tomato. The global production in 2017 was ca. 52 million t in ca. 1.8 million ha, 7% of the arable land destined to cultivate for this family, with an estimated value of $36.2B. Most of eggplants production is made in China and India, with ca. 87% of global production done there in 2017. Italy, Spain and Romania were the top three eggplant producer countries in the EU, with 286,473, 225,912 and 124,429 t produced, respectively (FAOSTAT 2017; Fig 7). Eggplant is not high in the majority of health-related micronutrients, but it is very low in fat an calorie and a rich source of nutritionally and pharmaceutically useful compounds, such a number phytonutrients, especially hydroxycinnamic acid (HCA) conjugates, potentially involved in consumer health, fruit taste and texture (Meyer et al. 2015; Chapman 2019).

Origin

The Asian eggplant is a widely grown species from the *Solanaceae* family. Eggplant is especially popular in the Southeast Asia and the Mediterranean region . Several non-exclusive theories have been proposed regarding eggplant's origin. The general consensus (Weese and Bohs 2010; Knapp et al. 2013) is that the African/Middle Eastern species *S. incanum* was transported into Indo-China where the true wild progenitor, *S. insanum*, evolved, from which *S. melongena* is derived (Chapman 2019).

Cultivation challenges

The commercial eggplant cultivars also have a narrow genetic diversity that, is even poorer than for other solanaceous crops such as tomato and pepper. A list of eggplant diseases and pests, and resistances described has been well summarized by Daunay (2008). Among the most common soil borne diseases affecting eggplants are bacterial, fusarium and verticillium wilt, caused by *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *melongenae*, *Verticillium dahliae* and *V. albo-atrum*. Moreover, it is also susceptible to RKN.

Grafting

Eggplant grafting is mostly used in intensive production conditions. As Daunay (2008) well summarize, there are currently three types of eggplant rootstock: i) *S. melongena* lines and hybrids that resist to Fusarium and bacterial wilt, and Phomopsis blight, ii) rootstocks based on the use of *S. integrifolium*, which also resists to Fusarium and bacterial wilts, and is used directly as a rootstock or as parent crossed with *S. melongena* varieties for producing interspecific hybrid rootstocks (*S. integrifolium* × *S. melongena*), and iii) the third type of rootstocks is composed by *Solanum* species such *S. torvum* and *S. sanitswongsei*. However,

within Europe eggplant is grafted mostly onto tomato or tomato interspecific hybrids (*L. esculentum* × *L. hirsutum*).

Solanum torvum is a wild relative of eggplant that is resistant to *V. dahliae*, *R. solanacearum*, *F. oxysporum* f. sp. *Melongenae*, and some RKN populations (Singh and Gopalakrishnan 1997; Bletsos et al. 2003; Daunay 2008; Gisbert et al. 2011). Although resistance of *S. torvum* rootstocks to *M. incognita* have been consistently described against several populations from France (Daunay and Dalmasso 1985), India (Shetty and Reddy 1985; Dhivya et al. 2014), Japan (Hara et al. 1983; Ali et al. 1992), Pakistan (Rahman et al. 2002) and Turkey (Çürük et al. 2009); several studies found discrepancies on the levels of resistance to *M. arenaria* (Daunay and Dalmasso 1985; Gonzalez et al; 2010; Ryu et al., 2011; Uehara et al. 2017b; Öçal et al. 2018) and *M. javanica* (Daunay and Dalmasso 1985; Boiteux and Charchar 1996; Tzortzakakis et al. 2006; Öçal et al. 2018). As far as literature reviewed pointed out, there are no enough studies that widely characterize the response of *S. torvum* against *Meloidogyne* populations occurring in Spain.

Most of grafting application have expanded mainly in the *Cucurbitaceae* and *Solanaceae* family, both major vegetable crops that are commonly rotated to maximize land use and boost productivity (López-Gómez et al. 2015; Kyriacou et al. 2017). The proper screening of *C. amarus* and *S. torvum* rootstocks could give crucial information about potential tools already available to design effective and environmentally friendlier strategies to managing RKN. In particular, this work will focus on *Meloidogyne* populations ocurrying in Spain, some of them previously described as virulents to the Mi1.2 gene in tomato (Ornat et al. 2007; Verdejo-Lucas et al. 2012).

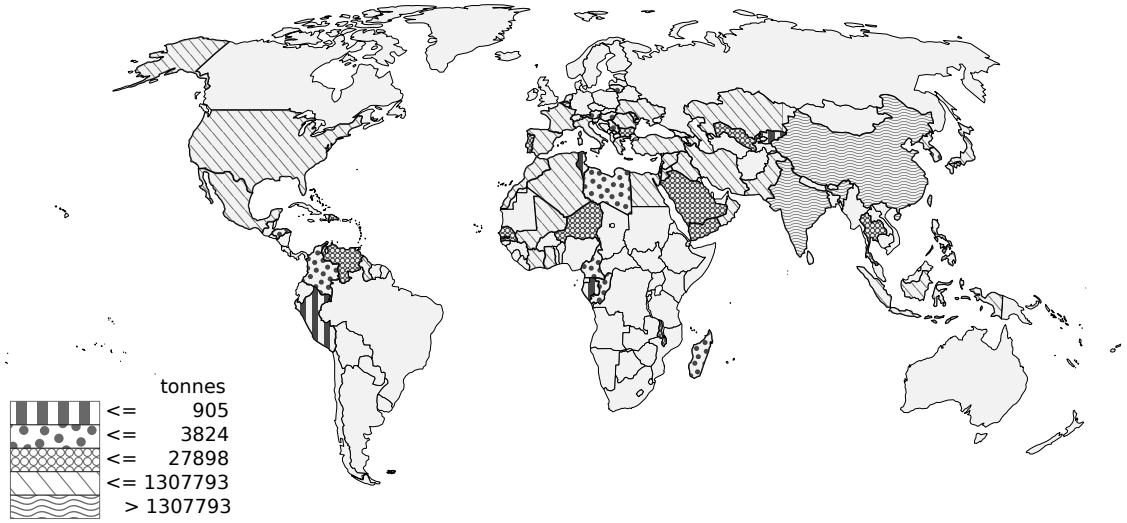


Figure 7: Production quantities of eggplants in 2017 by country (FAOSTAT, 2019).

Objectives

The main objective of this thesis was to determine the durability of resistance to *Meloidogyne* of *Citrullus amarus* and *Solanum torvum* as potential rootstocks for watermelon and eggplant, respectively. This objective was divided into specific objectives to determine:

1. The response of two *Citrullus amarus* accessions, BGV0005164 and BGV0005167, against different *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* (a)virulent isolates in pot experiments and against *M. incognita* in plastic greenhouse (Chapter 1).

2. The performance of ungrafted and grafted watermelon onto *C. amarus* accessions submitted to increasing densities of *M. incognita* and *M. javanica* in pot experiments to determine the maximum multiplication rate, the maximum population density and the equilibrium density of the root-knot nematode species and the effect on shoot dry biomass of watermelon (Chapter 2).

3. The durability of resistance of *C. amarus* accessions after two consecutive years in the same plots, the selection for virulence and the fitness cost for the nematode (Chapter 2).

4. The response of commercial *Solanum torvum* cultivars against (a)virulent isolates of *M. incognita* and *M. javanica* in pot experiments and against *M. incognita* in plastic greenhouse in two cropping seasons (Chapter 3).

5. The performance of ungrafted and grafted eggplant onto *S. torvum* 'Brutus' submitted to increasing densities of *M. javanica* in pot experiments to determine the maximum multiplication rate, the maximum population density and the equilibrium density of the root-knot nematode species and the effect on shoot dry biomass of watermelon (Chapter 3).

6. The durability of resistance of *S. torvum* 'Brutus' after two consecutive years in the same plots, the selection for virulence and the fitness cost for the nematode (Chapter 4).

Chapter 1

Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon

Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon

Helio A. García-Mendívil, María Munera, Ariadna Giné, Nuria Escudero, María Belén Picó, Carmina Gisbert and Francisco J. Sorribas.

2019. *Crop Protection*, 119, 208–213. https://doi.org/10.1016/j.cropro.2019.02.005

Abstract

The response of two *Citrullus amarus* accessions, BGV0005164 and BGV0005167, was assessed against different *Meloidogyne arenaria*, *Meloidogyne incognita*, and *Meloidogyne javanica* isolates in pot experiments and against *M. incognita* in plastic greenhouse. In the pot experiments, plants were inoculated with a second-stage juvenile per cm^3 of sterile sand and maintained in a growth chamber at 25 °C for 50 days. The watermelon cv. Sugar Baby was included as a susceptible control for comparison. At the end of the experiments, the number of egg masses and eggs per plant was determined, and the reproduction index was calculated as the percentage of the number of eggs produced in the *C. amarus* accessions with regard to that produced in the susceptible cv. Sugar Baby. In the plastic greenhouse experiment, the ungrafted watermelon cv. Sugar Baby and watermelons grafted onto each of the *C. amarus* accessions and onto the watermelon rootstock cv. Robusta were cultivated from May to August 2016 in plots with nematode densities from 46 to 1392 J2 per 250 cm^3 of soil at transplantation. At the end of the experiment, the galling index and the number of eggs per plant were determined, and the reproduction index was calculated. Additionally, the compatibility of the two accessions with the watermelon cv. Sugar Baby and the effect on fruit quality (weight, size, shape, firmness, pH, total soluble solids, and flesh color) were assessed under a hydroponic system in a greenhouse. The commercial rootstocks cv. Cobalt and cv. Robusta were also included. All the *Meloidogyne* isolates produced less egg masses and eggs per plant on the accessions than on Sugar Baby. Both accessions performed as resistant against *M. arenaria*, and from highly to moderately resistant to *M. incognita* and *M. javanica* in pot experiments. In the plastic greenhouse experiment, both *C. amarus* accessions performed as resistant to *M. incognita*. Both *C. amarus* accessions were compatible with the watermelon cv. Sugar Baby, but only the BGV0005167 accession did not influence the fruit quality. Then, the BGV0005167 accession is a promising rootstock for managing the three tropical root-knot nematode species without influencing watermelon fruit quality.

1.1 Introduction

Watermelon is one of the major cultivated cucurbit crops, with an estimated worldwide production of ca. 117 million t from 3.5 million ha (FAOSTAT 2017). As a result of the intensive cultivation in limited land resources, soilborne diseases and pests have significantly increased in recent years (Thies et al. 2015b). The root-knot nematode (RKN) *Meloidogyne* spp. is currently one of the main pathogens in cucurbit crops. Maximum yield losses of 88% in cucumber, 53% in zucchini, and 35% in watermelon cultivated under plastic greenhouses have been estimated in Spain (Giné et al. 2014; Vela et al. 2014; López-Gómez et al. 2014, 2015). The control of RKN has widely been done using fumigant and non fumigant nematicides (Nyczepir and Thomas 2009). Nonetheless,

the interest in nonchemical control alternatives has increased according to recent regulations such as the European Directive 2009/128/EC and the U.S. Clean Air Act (US Environmental Protection Agency 2012). In this scenario, plant resistance is a key tool for RKN management because it is an effective and economically profitable control method (Sorribas et al. 2005). Cropping resistant cultivars reduces the growth rate and the equilibrium density of the RKN population, as well as crop yield losses (Talavera et al. 2009). Moreover, it reduces crop yield losses of the following crop in the rotation scheme (Ornat et al. 1997; Thies et al. 2004; Westphal 2011). Grafting onto resistant rootstocks is an alternative method to control soilborne pathogens when no commercial resistant cultivars are available (Yetişir et al. 2003; Miguel et al. 2004; Cohen et al. 2007; Oda 2002; Thies et al. 2016). Regarding watermelon, it has been commonly grafted onto commercial rootstocks such as *Cucurbita maxima* × *Cucurbita moschata* and *Lagenaria siceraria* owing to their resistance to fusarium wilt. However, both rootstocks are susceptible to infection by *Meloidogyne* (Davis et al. 2008; Hassell et al. 2008; Thies et al. 2010, 2015a; Kokalis-Burelle and Rosskopf 2011; López-Gómez et al. 2016; Giné et al. 2017). In the last few years, some accessions of citron melon, *Citrullus lanatus* var. *citroides*, most recently referred as *Citrullus amarus* (Chomicki and Renner 2015), have been proven to be useful as watermelon rootstock. Indeed, these accessions provide resistance to fusarium wilt (Huitrón et al. 2007; Levi et al. 2017) and some RKN species in both greenhouse (Thies and Levi 2003, 2007) and open field cultivation (Huitrón et al. 2007; Thies et al. 2010, 2015a, 2016). In addition, watermelon grafted onto *C. amarus* yielded more than those grafted onto *L. siceraria*, *C. maxima* × *C. moschata* or *Praecitrullus fistulosus*, without affecting the quality and the size of the fruits (Kyriacou et al. 2016; Thies et al. 2015aa; Fredes et al. 2017). However, not all *C. amarus* accessions responded equally to RKN isolates (Thies and Levi 2003, 2007; Thies et al. 2016; Levi et al. 2017), the screening of new accessions against local RKN populations being necessary to assure their efficacy. Furthermore, the compatibility with the scion and the effect on the quality of fruits is also required to be considered as a potential rootstock. The aim of this study was to characterize the response of two experimental *C. amarus* accessions against several isolates of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* under controlled conditions and against *M. incognita* under plastic greenhouse conditions. Additionally, the compatibility of the two *C. amarus* accessions with the watermelon cv. Sugar Baby and the effect on fruit quality were assessed in a hydroponic system under greenhouse.

1.2 Materials and methods

1.2.1 Nematode inoculum

Seven isolates of *M. arenaria*, *M. incognita* and *M. javanica* were used in the experiments (Table 1.1). All the RKN isolates were maintained on the susceptible tomato cv. Durinta (Seminis Seeds, St. Louis, Missouri). Second-stage juveniles (J2) were used as the inoculum. The J2 were obtained from eggs by maceration of the infected roots in a 5% commercial bleach solution (40 g/L NaOCl) for 10 min according to the Hussey and Barker (1973) method. After maceration, the egg suspension was filtered through a 74 μm sieve, and then, the eggs were collected on a 25 μm sieve and placed on Baermann trays (Whitehead and Hemming 1965). The J2 emerged during the first 24 h were discarded. After that, the J2 emerged were recovered every two days and maintained at 9 °C until the pot experiments were carried out. The identification of the *Meloidogyne* species was confirmed using SCAR-PCR markers (Zijlstra et al. 2000).

Table 1.1: *Meloidogyne* species and isolates used in pot experiments, geographic origin, and (a)virulence status against the *Mi1.2* gene of tomato.

Meloidogyne spp.	Isolate	Geographic origin	(a)virulence	Reference
M. arenaria	MA68	Barcelona	Avirulent	Expósito et al. 2018
M. incognita	Agropolis	Barcelona	Avirulent	Giné et al. 2017
	Garriga	Barcelona	Avirulent	Expósito et al. 2018
M. javanica	MJ05	Barcelona	Avirulent	Ornat et al. 2007
	Tugues	Barcelona	Avirulent	Expósito et al. 2018
	Bay	Murcia	Avirulent	Expósito et al. 2018
	MJLg	Almería	Virulent	Expósito et al. 2018

1.2.2 Response of *C. amarus* accessions to RKN isolates

The *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), obtained from Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV-UPV) gene bank collection (Valencia, Spain), were assessed against the *Meloidogyne* isolates in three different pot experiments. In the first experiment, the accessions CI64 and CI67 were assessed against the *Mi1.2* avirulent isolates Agropolis (*M. incognita*) and MJ05 (*M. javanica*). In the second experiment, the response of the two *C. amarus* accessions was assessed against the *Mi1.2* avirulent isolates MA68 of *M. arenaria*; Agropolis and Garriga of *M. incognita*; and Bay, MJ05, and Tugues of *M. javanica*. In the third experiment, the response of both *C. amarus* accessions was assessed against the *Mi1.2* virulent isolate MJLg of *M. javanica*. The watermelon cv. Sugar Baby (SB) (Intersemillas S. A., Loriguilla, Valencia, Spain) was included as susceptible control for comparison in all experiments. The watermelon rootstock cv. Robusta (RO) (*C. lanatus*, Intersemillas S. A., Loriguilla, Valencia, Spain) was also included for comparison as resistant control (López-Gómez et al. 2016) in the third experiment. Experiment 1 and 3 were carried out once, and each plant-RKN isolate combination was replicated 10 times. Experiment 2 was repeated once, and each plant-RKN isolate combination was replicated seven and eight times in the first and second experiment repetition, respectively.

All experiments were carried out following the same procedure. Briefly, seeds were germinated according to the method given in Exposito et al. (2018). Seedlings were transplanted to 200 cm^3 pots containing sterile sand and maintained in a growth chamber at 25 ± 2°C with a 16:8 h (light:dark) photoperiod for a week and then inoculated with 1 J2 per cm^3 soil. Plants were maintained in the growth chamber for 50 days. Plants were watered as needed throughout the experiment and fertilized with a slow-release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with a PT100 probe (Campbell Scientific Ltd.) placed into the pots at 4 cm depth.

At the end of the experiments, the roots were carefully washed and weighed. Then, in the first and second experiments, the roots were submerged in 15 mg/L erioglaucine solution (Acros Organics) for 20 min to stain the egg masses before counting them (Omwega 1988). In all experiments, eggs were extracted from roots by maceration in a 10% commercial bleach solution (40 g/L NaOCl) for 10 min (Hussey and Barker 1973), passed through a 74 μm aperture screen and collected in a 25 μm sieve for final counting. Reproduction index (RI) was calculated as the percentage of eggs per plant produced in the experimental germplasm with regard to that in the susceptible one. The response of the accessions was categorized according to the RI as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI <

50%), or susceptible (RI $\geq$ 50%) (Hadisoeganda 1982).

1.2.3 Experiment under plastic greenhouse

The experiment was carried out from May 10 to August 11, 2016, under a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain), infested with the *M. incognita* isolate Agropolis. Ten 2.5 m long individual plots were used. Each plot was considered a replication and consisted in a row in which one plant each of ungrafted watermelon SB, the watermelon grafted onto CI64 and CI67, and that grafted onto the rootstock RO was transplanted with a space of 0.6 m. Plants were arranged in such a way that every germplasm was an equal number of times at the edge of the plots and next to the susceptible SB. Plants were irrigated as needed through a drip irrigation system and weekly fertilized with a solution consisting of NPK (15-5-30) at 31 kg/ha and iron chelate and micronutrients at 0.9 kg/ha. Plants were maintained for 20 weeks. The temperature was recorded at 30 min interval with temperature probes 5TM (Decagon Devices, Inc.) placed at a depth of 15 cm in the soil.

Nematode densities were determined at transplantation (Pi). Soil samples were taken from each experimental plot and consisted of eight cores taken from the first 30 cm of soil with an auger of diameter 2.5 cm. Soil subsamples were mixed and passed through a 4 mm pore sieve to remove stones. The J2 were extracted from 500 cm^3 of soil using Baermann trays (Whitehead and Hemming 1965) and incubated at 27 $\pm$ 2 °C for one week. Afterwards, the J2 were collected using a 25 μm aperture screen, counted, and expressed as J2 per 250 cm^3 of soil. At the end of the experiment, roots were carefully removed from the soil, washed, and weighed, and the galling index (GI) was evaluated on a scale from 0 to 10, where 0 complete and healthy root system and 10 plants and roots dead (Zeck 1971). After that, the number of eggs per plant was determined as described previously and was considered the final nematode density (Pf). RI was calculated and the response of the *C. amarus* accessions and RO was categorized as described previously.

1.2.4 Grafting compatibility and fruit quality

The watermelon cultivar SB was self-grafted (SB-SB) and grafted onto CI64, CI67, RO, and the commercial hybrid *C. maxima* $\times$ *C. moschata* rootstock cv. Cobalt (CO) (Rijk Zwaan, BV, The Netherlands) according to the cleft procedure (Lee et al., 2010). Ten plants of each grafted combination were grown under a hydroponic system in a commercial greenhouse at Fundación Cajamar (Paiporta, Valencia) during the spring–summer 2018. The ungrafted watermelon SB was included for comparison. To evaluate the impact of grafting on fruit quality, ten fruits per treatment were characterized for the following traits: weight, length and width, rind and flesh thickness, flesh firmness (measured with a digital Penetrometer (8 mm) FHT-803®, Melrose, MA), pH (measured with the pH indicator paper pH1-14; Merck, Darmstadt, Germany), total soluble solids (quantified using the digital refractometer Atago®, Tokyo, Japan), and flesh color (measured with the colorimeter Minolta CR-400, New Jersey, USA) using the color parameters Hunter L, a and b, where the L value indicates lightness (from 0 to 100), a value indicates redness (+) or greenness (−), and b value indicates yellowness (+) or blueness (−).

1.2.5 Statistical analysis

Statistical analyses were performed using R Statistical Software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The data on the number of egg masses and eggs per plant were not normally distributed according to the normal Shapiro–Wilk W test. Data from both repetitions of the second experiment were submitted to the nonparametric Mann–Whitney U test and pooled together as replications of the same experiment because no differences were found

($P \geq 0.05$). Comparisons between plant germplasm per each RKN isolate, as well as between RKN isolates per each plant germplasm within each experiment were done by the Mann–Whitney U test (two groups) or the Kruskal–Wallis non parametric test (more than two groups). When significant ($P < 0.05$), medians were separated using pairwise multiple comparisons by the Dunn test ($P < 0.05$). Data on fruit quality traits of each grafted combination were compared to those of the ungrafted control SB by the Student t-test ($P < 0.05$)

1.3 Results

1.3.1 Pot experiments

The number of egg masses and eggs per plant was lower ($P < 0.05$) in both *C. amarus* accessions than in the watermelon SB, irrespective of the RKN isolate. Both *C. amarus* accessions responded as resistant (1% $\leq$ RI <10%) to the majority of the RKN isolates. The accession CI64 responded only as moderately resistant to the *M. javanica* isolate Tugues, and both CI67 and RO were moderately resistant to the *Mi1.2* virulent MJLg isolate of *M. javanica* (Table 1.2).

1.3.2 Experiment under plastic greenhouse

The minimum and maximum soil temperatures during the experiment were 18.4 °C and 30.5 °C, respectively. The initial nematode densities at transplantation ranged from 46 to 1392 J2 per 250 cm^3 of soil. The number of eggs per plant and the galling index were significantly lower ($P < 0.05$) in both *C. amarus* accessions than those in the watermelon SB and the rootstock RO. Both CI accession and the rootstock RO performed as resistant (1% $\leq$ RI <10%) to *M. incognita* (Table 1.3).

1.3.3 Grafting compatibility and fruit quality

Under our experimental conditions, both ungrafted watermelon SB and watermelon SB grafted onto different rootstocks showed a similar growth performance. However, some effects of fruit traits were observed in plants grafted onto specific rootstocks (Table 1.4). The weight of watermelon fruits produced by SB onto the Cucurbita hybrid rootstock CO was greater ($P < 0.05$) than the weight of those produced by the ungrafted plants (5.5 $\pm$ 0.2 *vs.* 4.7 $\pm$ 0.5 kg) but with a significant decrease ($P < 0.05$) of soluble solids (9.45 $\pm$ 0.27 *vs.* 10.67 $\pm$ 0.32 °Bx). The watermelon rootstocks RO and CI67 did not influence the fruit traits compared to those produced by the ungrafted and self-grafted SB, but the rootstock CI64 produced fruits with thicker rinds, firmer flesh, and less soluble solids ($P < 0.05$) (Table 1.4).

Table 1.2: Number of egg masses and eggs per plant and reproduction index (RI) of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* isolates in the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), the watermelon cv. Sugar Baby (SB), and the commercial rootstock cv. Robusta (RO) 50 days after inoculation in pot experiments.

			Egg masses per plant			Eggs per plant($\times 10^2$)				RI (%)[a]		
Experiment	RKN species	Isolate	CI64	CI67	SB	CI64	CI67	SB	RO	CI64	CI67	RO
Experiment 1	*M. incognita*	Agropolis	1.0 ± 0.2 b A	1.0 ± 0.7 b A	17.0 ± 3.7 a A	3.5 ± 1.6 b A	4.6 ± 3.2 b A	126.9 ± 26.7 a A	2.7 ± 1.3	3.6 ± 2.5		
	M. javanica	MJ05	1.0 ± 0.6 b A	2.0 ± 0.7 b A	25.0 ± 0.2 a A	8.7 ± 8.2 b A	12.6 ± 11 b A	180.5 ± 32.5 a A	4.8 ± 4.5	7.0 ± 5.8		
Experiment 2	*M. arenaria*	MA68	0.8 ± 0.2 b A	0.5 ± 0.2 b A	5.0 ± 1.2 a A	2.1 ± 0.8 b A	1.1 ± 0.6 b A	39.8 ± 11.4 a A	5.3 ± 1.3	2.8 ± 1.5		
	M. incognita	Agropolis	0.5 ± 0.4 b AB	0.1 ± 0.1 b A	5.0 ± 1.6 a A	0.4 ± 0.4 b AB	0.1 ± 0.1 b A	23.9 ± 13.7 a AB	1.7 ± 1.5	0.5 ± 0.5		
		Garriga	0.3 ± 0.1 b AB	0.2 ± 0.1 b A	4.0 ± 0.9 a AB	0.1 ± 0.1 b B	0.3 ± 0.2 b A	12.2 ± 3.5 a AB	0.5 ± 0.3	2.6 ± 1.7		
	M. javanica	Bay	0.3 ± 0.1 b AB	0.6 ± 0.2 b A	6.0 ± 1.7 a A	0.2 ± 0.1 b AB	0.3 ± 0.1 b A	33.8 ± 12.0 a A	0.5 ± 0.3	0.9 ± 0.4		
		MJ05	0.7 ± 0.2 b AB	0.4 ± 0.2 b A	5.0 ± 1.0 a A	1.2 ± 0.8 b AB	1.0 ± 0.8 b A	30.0 ± 9.1 a AB	4.0 ± 2.5	3.2 ± 2.8		
		Tugues	0.1 ± 0.1 b B	0.4 ± 0.2 b A	1.0 ± 0.1 a B	0.5 ± 0.5 b B	0.3 ± 0.1 b A	3.7 ± 1.7 a B	14.3 ± 13.5	7.2 ± 3.6		
Experiment 3	*M. javanica*	MJLg	na	na	na	0.3 ± 0.3 b	0.7 ± 0.6 b	6.6 ± 2.4 a	1.2 ± 0.5 ab	5.0 ± 5.0	10.4 ± 8.8	17.5 ± 8.1

Data are the mean ± standard error of 10 replicates in experiments 1 and 3 and of 15 replicates in experiment 2. Data within the same row followed by the same lower case letter did not show significant difference ($P < 0.05$) between germplasm per RKN isolate according to the Kruskal–Wallis test. Different capital letters in the same column and experiment indicate significant differences ($P < 0.05$) between nematode isolates according to the Mann–Whitney U test (experiment 1) or Kruskal–Wallis test (experiment 2); na: not assessed.

[a]Reproduction index = 100 × (number of eggs per plant produced in the CI accessions or RO / mean number of eggs per plant produced in the susceptible cv. Sugar Baby).

Table 1.3: Galling index, eggs per plant and reproduction index (RI) of *M. incognita* in the watermelon cv. Sugar Baby, the commercial watermelon rootstock cv. Robusta, and the *C. amarus* accessions BGV0005164 and BGV0005167 cultivated from May to August 2016 in plastic greenhouse at initial population densities from 46 to 1392 J2 per 250 cm^3 of soil.

Plant host	Galling index[a]	Eggs per plant ($\times 10^2$)	Reproduction index (%)[b]
Sugar Baby	5.0 ± 0.6 a	1031 ± 484 a	
Robusta	2.8 ± 0.4 b	51 ± 11 b	4.4 ± 0.9
BGV0005164	2.5 ± 0.5 b	16 ± 10 b	1.4 ± 0.9
BGV0005167	1.5 ± 0.5 b	15 ± 12 b	1.3 ± 1.0

Data are the mean ± standard error of 10 replicates. Different letters in the same column indicate significant differences ($P < 0.05$) between germplasm according to the Kruskal–Wallis test.

[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck 1971).

[b]Reproduction index = 100 × (number of eggs per plant produced in the CI accessions or Robusta / mean number of eggs per plant produced in the susceptible cv. Sugar Baby).

1.4 Discussion

The results of this study showed that the *C. amarus* accessions CI64 and CI67 are resistant to several nematode isolates belonging to the three most widespread RKN species. Some other *C. amarus* accessions resistant to RKN have been reported previously (Huitrón et al. 2007; Thies et al. 2015c), thus increasing the number of accessions that could be used as putative watermelon rootstock. The watermelon has been described as a poor host of *Meloidogyne* owing to its low values of maximum multiplication rate and equilibrium density (López-Gómez et al. 2014). The RKN isolates assessed in this study reproduced less than watermelon cv. Sugar Baby in both pot and plastic greenhouse experiments, which demonstrates their potential for suppressing the RKN population growth rate. Other *C. amarus* accessions and lines have also been shown to be RKN resistant under field and plastic greenhouse conditions (Huitrón et al. 2007; Thies et al. 2008, 2009, 2015a, 2015b, 2015c). The resistance of *C. amarus* to RKN has been associated with the relatively high root fibrosity compared to that of *C. lanatus* var. *lanatus*, *Citrullus colocynthis*, *L. siceraria*, and *C. maxima* × *C. moschata* (Thies and Levi 2003, 2007; Thies et al. 2015c, 2016).

Interestingly, both *C. amarus* accessions assessed in this study were also resistant to a *Mi1.2* gene virulent isolate. This finding shows the usefulness to include this germplasm as a component of the rotation scheme for managing virulent RKN isolates for specific resistance genes. The most available resistance genes to RKN in vegetables are in solanaceous cultivars and rootstocks, e.g., tomato and pepper. The virulence to a given *R*-gene could be counter-selected by other *R*-genes because it is highly specific and it has a fitness cost to be acquired (Djian-Caporalino et al. 2011). Recently, some *Cucumis metuliferus* accessions have been described as resistant to *Mi1.2* gene virulent RKN isolates (Expósito et al. 2018), and although the selection for virulence to the *Mi1.2* gene was not prevented when alternated with tomato grafted onto the resistant rootstock cv. Aligator, it influenced its level (Expósito et al. 2019). The availability of some more sources of resistance used in rotation schemes could favor the durability of specific resistant genes by preventing the fixation of the virulence character in the RKN population.

Grafting commercial watermelon cultivars onto resistant rootstocks has proven to be a successful approach to manage plant diseases, being a widely accepted practice in some parts of the world (Oda 2002; Miguel

Table 1.4: Number of egg masses and eggs per plant and reproduction index (RI) of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* isolates in the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), the watermelon cv. Sugar Baby (SB), and the commercial rootstock cv. Robusta (RO) 50 days after inoculation in pot experiments.

Rootstock-scion	Fruit size			Rind thickness (mm)	Flesh thickness (cm)	Flesh firmness (kg/cm^2)	Soluble solid (Brix°)		Color[b]		
	Weight (kg)	Length (cm)	Width (cm)					pH	*L*	*a*	*b*
SB	4.7 ± 0.5a	20.33 ± 0.54	21.10 ± 0.44	11.03 ± 1.07	18.77 ± 0.11	1.33 ± 0.16	10.67 ± 0.32	5.21 ±0.19	33.38 ± 1.35	18.58 ± 1.24	12.03 ± 0.34
SB-SB	5.0 ± 0.3	20.96 ± 0.38	21.47 ± 0.23	11.25 ± 0.78	18.87 ± 0.27	1.52 ± 0.14	10.03 ± 0.23	5.00 ± 0.11	30.09 ± 1.61	18.30 ± 0.87	11.00 ± 1.01
CO-SB	5.5 ± 0.2*	21.25 ± 0.47	21.95 ± 0.38	11.12 ± 0.92	19.50 ± 0.35	1.75 ± 0.14	9.45 ± 0.27*	5.38 ± 0.16	32.91 ± 1.17	20.63 ± 1.07	12.05 ± 0.54
RO-SB	5.1 ± 0.3	20.85 ± 0.38	21.32 ± 0.33	12.92 ± 0.73	18.65 ± 0.27	1.62 ± 0.11	10.02 ± 0.22	5.0 ± 0.17	31.29 ± 0.78	18.94 ± 0.38	11.49 ± 0.33
CI64-SB	5.2 ± 0.2	21.42 ± 0.38	21.55 ± 0.31	14.04 ± 0.75*	18.4 ± 0.29	1.76 ± 0.12*	9.77 ± 0.22*	5.00 ± 0.13	31.94 ± 0.96	19.08 ± 0.87	11.98 ± 0.44
CI67-SB	5.0 ± 0.2	20.93 ± 0.31	21.38 ± 0.25	12.89 ± 0.62	18.54 ± 0.24	1.62 ± 0.09	10.3 ± 0.18	5.17 ± 0.13	32.80 ±0.88	19.03 ± 0.71	12.18 ± 0.36

[a]Data are the mean ± standard error of 10 replicates. Data in the same column followed by * indicate significant differences ($P < 0.05$) with regard to the ungrafted watermelon cv. Sugar Baby (SB) according to Student's t-test.
[b]Color: parameters measured in fruit flesh: Hunter *L*, lightness (from 0 to 100); *a*, red (+); *b*, yellow (+) or blue (−).

et al. 2004; Cohen et al. 2007; Yetişir et al. 2007; Leonardi et al. 2017). Cucurbita hybrids, the most popular watermelon rootstocks, are resistant to some soil-borne fungal diseases but susceptible to RKN (López-Gómez et al. 2016; Giné et al. 2017). The results of this study showed that both *C. amarus* accessions are able to suppress RKN at the same level as that of the commercial *C. lanatus* cv. Robusta. In addition, these two experimental accessions have also been proved to be moderately to highly resistant to *Fusarium oxysporum* f. sp. *niveum* (Fon) races 0 and 2 (Garces et al., personal communication), which improve their success as watermelon rootstock. Some other *C. amarus* accessions also showed resistance to other diseases such as gummy stem blight (Gusmini et al. 2005), powdery mildew (Davis et al. 2007; Tetteh et al. 2010), and potyviruses (Guner 2004; Strange et al. 2002; Guner and Wehner 2008)

Both *C. amarus* accessions have shown efficient grafting compatibility to watermelon, but they differed in influencing the fruit quality. While the quality of fruit produced by the watermelon grafted onto the CI67 accession did not show significant difference from that produced by the ungrafted and self-grafted plants, it did show a significant difference when grafted onto CI64. Similar results were obtained with the watermelon F1 hybrid cv. Oneida onto CI67 (Fredes et al. 2017). This previous study also showed that the citron melon accession affected the aroma of the watermelon flesh less than the hybrid Cucurbita rootstock, which, in turn, produced larger fruit with less soluble solids.

1.5 Conclusion

The *C. amarus* accession CI67 is a promising rootstock for managing the three tropical RKN species without influencing watermelon fruit quality.

Acknowledgments

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R, AGL2014-53398-C2-2-R-AR, AGL2017-89785-R, and AGL2017-85563-C2-1-R-AR. H. A. García-Mendívil acknowledges CONACYT for suport through a predoctoral grant.

References

Chomicki, G., and S. S. Renner. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. *New Phytologist* 205, no. 2 (): 526–532. ISSN: 0028646X. doi:10.1111/nph.13163. http://doi.wiley.com/10.1111/nph.13163.

Cohen, R., Y. Burger, C. Horev, A. Koren, and M. Edelstein. 2007. Introducing Grafted Cucurbits to Modern Agriculture: The Israeli Experience. *Plant Disease* 91, no. 8 (): 916–923. ISSN: 0191-2917. doi:10.1094/PDIS-91-8-0916. http://apsjournals.apsnet.org/doi/10.1094/PDIS-91-8-0916.

Davis, A. R., A. Levi, A. Tetteh, T. Wehner, V. Russo, and M. Pitrat. 2007. Evaluation of Watermelon and Related Species for Resistance to Race 1W Powdery Mildew. *Journal of the American Society for Horticultural Science* 132, no. 6 (): 790–795. ISSN: 0003-1062. doi:10.21273/JASHS.132.6.790. https://journals.ashs.org/view/journals/jashs/132/6/article-p790.xml.

Davis, A. R., P. Perkins-Veazie, Y. Sakata, S. López-Galarza, J. V. Maroto, S.-G. Lee, et al. 2008. Cucurbit Grafting. *Critical Reviews in Plant Sciences* 27, no. 1 (): 50–74. ISSN: 0735-2689. doi:10.1080/07352680802053940. http://www.tandfonline.com/doi/abs/10.1080/07352680802053940.

Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, et al. 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *European Journal of Plant Pathology* 131, no. 3 (): 431–440. ISSN: 0929-1873. doi:10.1007/s10658-011-9820-4. http://link.springer.com/10.1007/s10658-011-9820-4.

Expósito, A., S. García, A. Giné, N. Escudero, and F. J. Sorribas. 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato–melon rotation sequence. *Pest Management Science* 75, no. 7 (): 1902–1910. ISSN: 1526-498X. doi:10.1002/ps.5297. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.5297.

Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, M. B. Picó, et al. 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67, no. 5 (): 1161–1167. ISSN: 00320862. doi:10.1111/ppa.12815. http://doi.wiley.com/10.1111/ppa.12815.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Statistics Division. Visited on 09/04/2019. http://www.fao.org/faostat.

Fredes, A., S. Roselló, J. Beltrán, J. Cebolla-Cornejo, A. Pérez-de-Castro, C. Gisbert, et al. 2017. Fruit quality assessment of watermelons grafted onto citron melon rootstock. *Journal of the Science of Food and Agriculture* 97, no. 5 (): 1646–1655. ISSN: 00225142. doi:10.1002/jsfa.7915. http://doi.wiley.com/10.1002/jsfa.7915.

Giné, A., C. González, L. Serrano, and F. J. Sorribas. 2017. Population dynamics of *Meloidogyne incognita* on cucumber grafted onto the Cucurbita hybrid RS841 or ungrafted and yield losses under protected cultivation. *European Journal of Plant Pathology* 148, no. 4 (): 795–805. ISSN: 0929-1873. doi:10.1007/s10658-016-1135-z. http://link.springer.com/10.1007/s10658-016-1135-z.

Giné, A., M. López-Gómez, M. D. Vela, C. Ornat, M. Talavera, S. Verdejo-Lucas, et al. 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology* 63, no. 6 (): 1446–1453. ISSN: 00320862. doi:10.1111/ppa.12217. http://doi.wiley.com/10.1111/ppa.12217.

Guner, N. 2004. Papaya Ringspot Virus Watermelon Strain and Zucchini Yellow Mosaic Virus Resistance in Watermelon. PhD thesis, North Carolina State University. http://www.lib.ncsu.edu/resolver/1840.16/3271.

Guner, N., and T. C. Wehner. 2008. Overview of Potyvirus resistance in watermelon. *Journal of Heredity*. https://w3.avignon.inra.fr/dspace/handle/2174/245.

Gusmini, G., R. Song, and T. C. Wehner. 2005. New Sources of Resistance to Gummy Stem Blight in Watermelon. *Crop Science* 45 (2): 582. ISSN: 1435-0653. doi:10.2135/cropsci2005.0582. https://www.crops.org/publications/cs/abstracts/45/2/0582.

Hadisoeganda, W. W. 1982. Resistance of Tomato, Bean, Southern Pea, and Garden Pea Cultivars to Root-Knot Nematodes Based on Host Suitability. *Plant Disease* 66 (1): 145. ISSN: 0191-2917. doi:10.1094/PD-66-145. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1982Abstracts/PD%7B%5C_%7D66%7B%5C_%7D145.htm.

Hassell, R. L., F. Memmott, and D. G. Liere. 2008. Grafting Methods for Watermelon Production. *HortScience* 43, no. 6 (): 1677–1679. ISSN: 0018-5345. doi:10.21273/HORTSCI.43.6.1677. https://journals.ashs.org/view/journals/hortsci/43/6/article-p1677.xml.

Huitrón, M. V., M. Diaz, F. Diánez, and F. Camacho. 2007. The effect of various rootstocks on triploid watermelon yield and quality. *Journal of Food, Agriculture and Environment*. ISSN: 14590263. https://pubag.nal.usda.gov/catalog/723527.

Hussey, R. S., and K. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025–1028.

Kokalis-Burelle, N., and E. N. Rosskopf. 2011. Microplot Evaluation of Rootstocks for Control of *Meloidogyne incognita* on Grafted Tomato, Muskmelon, and Watermelon. *Journal of nematology* 43, **numbers** 3-4 (): 166–71. ISSN: 0022-300X. http://www.ncbi.nlm.nih.gov/pubmed/23431109%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3547354.

Kyriacou, M. C., G. A. Soteriou, Y. Rouphael, A. S. Siomos, and D. Gerasopoulos. 2016. Configuration of watermelon fruit quality in response to rootstock-mediated harvest maturity and postharvest storage. *Journal of the Science of Food and Agriculture*. ISSN: 10970010. doi:10.1002/jsfa.7356.

Leonardi, C., M. C. Kyriacou, C. Gisbert, G. B. Oztekin, I. Mourão, and Y. Rouphael. 2017. Quality of grafted vegetables. In *Vegetable grafting: principles and practices*, 216–244. Wallingford: CABI. doi:10.1079/9781780648972.0216. http://www.cabi.org/cabebooks/ebook/20173181047.

Levi, A., R. Jarret, S. Kousik, W. Patrick Wechter, P. Nimmakayala, and U. K. Reddy. 2017. Genetic Resources of Watermelon. In *Plant Genetics and Genomics: Crops and Models*, 87–110. Springer International. doi:10.1007/7397_2016_34. http://link.springer.com/10.1007/7397%7B%5C_%7D2016%7B%5C_%7D34.

López-Gómez, M., E. Flor-Peregrín, M. Talavera, F. J. Sorribas, and S. Verdejo-Lucas. 2015. Population dynamics of *Meloidogyne javanica* and its relationship with the leaf chlorophyll content in zucchini. *Crop Protection* 70 (): 8–14. ISSN: 02612194. doi:10.1016/j.cropro.2014.12.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219414003871.

López-Gómez, M., A. Giné, M. D. Vela, C. Ornat, F. J. Sorribas, M. Talavera, et al. 2014. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. *Annals of Applied Biology* 165, no. 3 (): 466–473. ISSN: 00034746. doi:10.1111/aab.12154. http://doi.wiley.com/10.1111/aab.12154.

López-Gómez, M., M. Talavera, and S. Verdejo-Lucas. 2016. Differential reproduction of *Meloidogyne incognita* and *M. javanica* in watermelon cultivars and cucurbit rootstocks. *Plant Pathology* 65, no. 1 (): 145–153. ISSN: 13653059. doi:10.1111/ppa.12394. http://doi.wiley.com/10.1111/ppa.12394.

Miguel, A., J. V. Maroto, A. San Bautista, C. Baixauli, V. Cebolla, B. Pascual, et al. 2004. The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. *Scientia Horticulturae* 103, no. 1 (): 9–17. ISSN: 03044238. doi:10.1016/j.scienta.2004.04.007. https://linkinghub.elsevier.com/retrieve/pii/S030442380400086X.

Nyczepir, A. P., and S. H. Thomas. 2009. Current and future management strategies in intensive crop production systems. In *Root-knot nematodes*, ed. by R. Perry, M. Moens, and J. Starr, 412–443. Wallingford: CABI. doi:10.1079/9781845934927.0412. http://www.cabi.org/cabebooks/ebook/20093330198.

Oda, M. 2002. Grafting of vegetable crops. *Scientific Report of Agricultural and Biological Science*. https://www.cabdirect.org/cabdirect/abstract/20023124609.

Omwega, C. O. 1988. A Nondestructive Technique for Screening Bean Germ Plasm for Resistance to *Meloidogyne incognita*. *Plant Disease* 72 (11): 970. ISSN: 0191-2917. doi:10.1094/PD-72-0970. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1988Abstracts/PD%7B%5C_%7D72%7B%5C_%7D970.htm.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 2007. A Population of *Meloidogyne javanica* in Spain Virulent to the *Mi* Resistance Gene in Tomato. *Plant Disease* 85, no. 3 (): 271–276. ISSN: 0191-2917. doi:10.1094/PDIS.2001.85.3.271. http://apsjournals.apsnet.org/doi/10.1094/PDIS.2001.85.3.271.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 1997. Effect of the previous crop on population densities of *Meloidogyne javanica* and yield of cucumber. *Nematropica*. ISSN: 0099-5444.

Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano, and J. Valero. 2005. Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology* 111, no. 1 (): 29–38. ISSN: 0929-1873. doi:10.1007/s10658-004-1982-x. http://link.springer.com/10.1007/s10658-004-1982-x.

Strange, E. B., N. Guner, Z. Pesic-VanEsbroeck, and T. C. Wehner. 2002. Screening the Watermelon Germplasm Collection for Resistance to Papaya Ringspot Virus Type-W. *Crop Science* 42 (4): 1324. ISSN: 1435-0653. doi:10.2135/cropsci2002.1324. https://www.crops.org/publications/cs/abstracts/42/4/1324.

Talavera, M., S. Verdejo-Lucas, C. Ornat, J. Torres, M. D. Vela, F. J. Macias, et al. 2009. Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses. *Crop Protection* 28, no. 8 (): 662–667. ISSN: 02612194. doi:10.1016/j.cropro.2009.03.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219409000763.

Tetteh, A. Y., T. C. Wehner, and A. R. Davis. 2010. Identifying Resistance to Powdery Mildew Race 2W in the USDA-ARS Watermelon Germplasm Collection. *Crop Science* 50 (3): 933. ISSN: 1435-0653. doi:10.2135/cropsci2009.03.0135. https://www.crops.org/publications/cs/abstracts/50/3/933.

Thies, J. A., J. J. Ariss, R. L. Hassell, S. Buckner, and A. Levi. 2015a. Accessions of *Citrullus lanatus* var. *citroides* Are Valuable Rootstocks for Grafted Watermelon in Fields Infested with Root-knot Nematodes. *HortScience* 50, no. 1 (): 4–8. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.4. https://journals.ashs.org/view/journals/hortsci/50/1/article-p4.xml.

Thies, J. A., J. J. Ariss, R. L. Hassell, and S. Olson. 2009. Grafting for Management of Root-Knot Nematodes in Watermelon. *HortScience* 44 (3): 576. https://www.ars.usda.gov/research/publications/publication/?seqNo115=224817.

Thies, J. A., J. J. Ariss, R. L. Hassell, S. Olson, C. S. Kousik, and A. Levi. 2010. Grafting for Management of Southern Root-Knot Nematode, *Meloidogyne incognita*, in Watermelon. *Plant Disease* 94, no. 10 (): 1195–1199. ISSN: 0191-2917. doi:10.1094/PDIS-09-09-0640. http://apsjournals.apsnet.org/doi/10.1094/PDIS-09-09-0640.

Thies, J. A., J. J. Ariss, C. S. Kousik, and R. L. Hassell. 2008. Grafting–a tool for managing root-knot nematodes in watermelon. *Phytopathology* 98:S156. https://www.ars.usda.gov/research/publications/publication/?seqNo115=224817.

Thies, J. A., J. J. Ariss, C. S. Kousik, R. L. Hassell, and A. Levi. 2016. Resistance to Southern Root-knot Nematode (*Meloidogyne incognita*) in Wild Watermelon (*Citrullus lanatus* var. *citroides*). *Journal of Nematology* 48 (1): 14–19. ISSN: 0022-300X. doi:10.21307/jofnem-2017-004. https://www.exeley.com/journal%7B%5C_%7Dof%7B%5C_%7Dnematology/doi/10.21307/jofnem-2017-004.

Thies, J. A., S. Buckner, M. Horry, R. Hassell, and A. Levi. 2015b. Influence of *Citrullus lanatus* var. *citroides* Rootstocks and Their F1 Hybrids on Yield and Response to Root-knot Nematode, *Meloidogyne incognita*, in Grafted Watermelon. *HortScience* 50, no. 1 (): 9–12. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.9. https://journals.ashs.org/view/journals/hortsci/50/1/article-p9.xml.

Thies, J. A., R. F. Davis, J. D. Mueller, R. L. Fery, D. B. Langston, and G. Miller. 2004. Double-Cropping Cucumbers and Squash After Resistant Bell Pepper for Root-Knot Nematode Management. *Plant Disease* 88, no. 6 (): 589–593. ISSN: 0191-2917. doi:10.1094/PDIS.2004.88.6.589. http://apsjournals.apsnet.org/doi/10.1094/PDIS.2004.88.6.589.

Thies, J. A., and A. Levi. 2003. Resistance of Watermelon Germplasm to the Peanut Root-knot Nematode. *HortScience* 38, no. 7 (): 1417–1421. ISSN: 0018-5345. doi:10.21273/HORTSCI.38.7.1417. https://journals.ashs.org/view/journals/hortsci/38/7/article-p1417.xml.

— . 2007. Characterization of Watermelon (*Citrullus lanatus* var. *citroides*) Germplasm for Resistance to Root-knot Nematodes. *HortScience* 42, no. 7 (): 1530–1533. ISSN: 0018-5345. doi:10.21273/HORTSCI.42.7.1530. https://journals.ashs.org/view/journals/hortsci/42/7/article-p1530.xml.

Thies, J. A., A. Levi, J. J. Ariss, and R. L. Hassell. 2015c. RKVL-318, a Root-knot Nematode-resistant Watermelon Line as Rootstock for Grafted Watermelon. *HortScience* 50, no. 1 (): 141–142. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.141. https://journals.ashs.org/view/journals/hortsci/50/1/article-p141.xml.

US Environmental Protection Agency. 2012. Ozone Layer Protection. Visited on 09/04/2019. https://www.epa.gov/ozone-layer-protection/.

Vela, M. D., A. Giné, M. López-Gómez, F. J. Sorribas, C. Ornat, S. Verdejo-Lucas, et al. 2014. Thermal time requirements of root-knot nematodes on zucchini-squash and population dynamics with associated yield losses on spring and autumn cropping cycles. *European Journal of Plant Pathology* 140, no. 3 (): 481–490. ISSN: 0929-1873. doi:10.1007/s10658-014-0482-x. http://link.springer.com/10.1007/s10658-014-0482-x.

Westphal, A. 2011. Sustainable approaches to the management of plant-parasitic nematodes and disease complexes. *Journal of Nematology* 43 (2): 122–125. ISSN: 0022-300X. https://journals.flvc.org/jon/article/view/78315.

Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, no. 1 (): 25–38. ISSN: 0003-4746. doi:10.1111/j.1744-7348.1965.tb07864.x. http://doi.wiley.com/10.1111/j.1744-7348.1965.tb07864.x.

Yetişir, H., Ş. Kurt, N. Sari, and F. M. Tok. 2007. Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: Plant growth, graft compatibility, and resistance to Fusarium. *Turkish Journal of Agriculture and Forestry*. ISSN: 1300011X. https://dergipark.org.tr/tbtkagriculture/issue/11612/138378.

Yetişir, H., N. Sari, and S. Yücel. 2003. Rootstock resistance to Fusarium wilt and effect on watermelon fruit yield and quality. *Phytoparasitica* 31, no. 2 (): 163–169. ISSN: 0334-2123. doi:10.1007/BF02980786. http://link.springer.com/10.1007/BF02980786.

Zeck, W. M. 1971. Rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschutz nachrichten*, no. 24: 141–144. ISSN: 0079-1342.

Zijlstra, C., D. T. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2 (8): 847–853. ISSN: 1388-5545. doi:10.1163/156854100750112798. https://brill.com/view/journals/nemy/2/8/article-p847%7B%5C_%7D3.xml.

Chapter 2

Effect of *Citrullus amarus* accessions on the population dynamics of *Meloidogyne incognita* and *M. javanica* and watermelon yield

Effect of *Citrullus amarus* accessions on the population dynamics of *Meloidogyne incognita* and *M. javanica* and watermelon yield

Helio A. García-Mendívil and Francisco J. Sorribas

Abstract

The response of ungrafted and grafted watermelon cv. Sugar baby onto the *C. amarus* accessions BGV0005164 and BGV0005167 submitted to increasing densities of *M. incognita* and *M. javanica* was studied in pot experiments to determine the maximum multiplication rate, the maximum population density and the equilibrium density of the root-knot nematode species and the effect on shoot dry biomass of watermelon. In plastic greenhouse conditions, the ungrafted and grafted watermelon onto both *C. amarus* accessions, and onto the *C. lanatus* rootstock cv. Robusta were cultivated for two consecutive years in the same plots to assess the level of resistance to *M. incognita* and crop yield. Additionally, after the second crop, the putative selection for virulence in the nematode subpopulation originated in the ungrafted and grafted watermelon was assessed in pot experiments. The maximum multiplication rate, the maximum population density and the equilibrium density values of both *Meloidogyne* species were lower in grafted than ungrafted watermelon. In the plastic greenhouse experiment, the nematode densities in soil at transplantation ranged from 1 to 53 J2 per 100 cm^3 of soil in 2017 and did not differ between grafted and ungrafted watermelons. At the end of the crop, the galling index and the number of eggs per plant was higher in ungrafted than in grafted watermelon both years. The *C. amarus* accessions performed from highly resistant to resistant to *M. incognita*, and the rootstock cv. Robusta from moderately resistant in 2017 to slightly resistant in 2018. The repeated cultivation of grafted watermelon onto *C. amarus* accessions did not select for virulence. All grafted watermelons yielded more kg per plant than the ungrafted in both years. The results of this study highlight the poorer host status of CI64 and CI67 accessions to *M. incognita* and *M. javanica* compared to watermelon; the stability of the *C. amarus* resistance; and the beneficial effect of *C. amarus* on watermelon yield when cultivated in *Meloidogyne* infested soils.

2.1 Introduction

Grafting vegetables onto resistant rootstocks for managing soilborne plant pathogens has become a common environmentally friendly alternative to soil fumigants (Thies et al. 2015b). Watermelon, the most cultivated cucurbit crop worldwide (FAOSTAT 2017), is commonly grafted onto *Cucurbita maxima* × *Cucurbita moschata* and *Lagenaria siceraria* rootstocks to control fusarium wilt (Miguel et al. 2004), but these rootstocks are susceptible to root-knot nematodes (RKN), *Meloidogyne* spp. (Huitrón et al. 2007; López-Gómez et al. 2016; Levi et al. 2017). Despite RKN are the main plant parasitic nematode affecting cucurbit crops (Hallmann and Meressa 2018), watermelon is less affected than others, such as cucumber or melon (Giné et al. 2014; Expósito et al. 2019), due to its poor host status (López-Gómez et al. 2014). Nonetheless, grafting onto interspecific squash hybrid rootstocks leads to increasing nematode densities (Giné et al. 2017) making necessary the use of other methods for RKN

management. Then, screening for other putative watermelon rootstocks resistant to both fusarium wilt and RKN, including *Citrullus amarus*, has been done (Thies and Levi 2003, 2007; Huitrón et al. 2007; Thies et al. 2010, 2015a, 2015, 2016; Keinath et al. 2019). As a result of these studies, the *C. amarus* cv. Carolina Strongback has been jointly released by USDA-ARS and Clemson University (Kemble et al. 2019). Recently, García-Mendívil et al. (2019) have reported two *C. amarus* accessions resistant to fusarium wilt that were also resistant to isolates of the three most widespread RKN species, *M. arenaria*, *M. incognita* and *M. javanica*, and were compatible as rootstocks for a watermelon cultivar. Nonetheless, a wider characterization of the potential of these plant germplasms for RKN management should consider the plant host status and the level of plant resistance, and also the plant tolerance to estimate yield losses. The host status of a plant is determined by three parameters estimated with the relationship between the nematode density at transplanting (Pi) and at the end of the crop (Pf): the maximum multiplication rate (a), the maximum population density (M), and the equilibrium density (E, $Pf = Pi$; $Pf/Pi = 1$), that are higher in good host than in resistant or poor host plants (Seinhorst 1970). The resistance level of a given plant can be categorized according to the reproduction index of the nematode, defined as the proportion of the nematode reproduction in a given germplasm compared to that in a susceptible standard (Hadisoeganda 1982). Plant tolerance (T), the maximum Pi at which no yield loss is recorded, is estimated by the relationship between increasing Pi and the relative plant biomass or crop yield. This parameter, along with the minimum relative yield (m), can be estimated by the Seinhorst's damage function model (Seinhorst 1998). In addition to this characterization, the knowledge of the risk of selection of virulent nematode populations can aid for designing good plant resistance management practices for preserving its durability. Then, several experiments were conducted to estimate the population dynamics of *M. incognita* and *M. javanica* on ungrafted and grafted watermelon onto two *C. amarus* accessions, its level of resistance and tolerance, and the effect of cultivation two consecutive watermelon crops in the same plots under plastic greenhouse conditions on *M. incognita* densities, selection for nematode virulence, and watermelon yield.

2.2 Materials and methods

2.2.1 Nematode inoculum

The isolates Agropolis of *M. incognita* and MJ05 of *M. javanica* were used in the experiments. Both RKN isolates were maintained on the susceptible tomato cv. Durinta (Seminis Seeds, St. Louis, Missouri). The nematode inoculum consisted in second-stage juveniles (J2) obtained from eggs by blender maceration of infected roots in a 5% commercial bleach solution (40 g/L NaOCl) for 10 min according to the Hussey and Barker (1973) method. Afterwards, the egg suspension was firstly filtered through a 74 μm sieve and finally collected on a 25 μm sieve and placed on Baermann trays (Whitehead and Hemming 1965). The J2 emerged during the first 24 h were discarded. After that, the J2 emerged were recovered every two days and maintained at 9 °C for 8 days until the pot experiments were carried out. The identification of the *Meloidogyne* species was confirmed using SCAR-PCR markers (Zijlstra et al. 2000)

2.2.2 Relationship between increasing *Pi* of *M. incognita* or *M. javanica* on ungrafted and grafted watermelon and *Pf* and plant biomass

The *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), provided by Dr. Gisbert and Dr. Picó from the Institute for the Conservation and Breeding of Agricultural Biodiversity gene bank collection (COMAV-UPV, Valencia, Spain), and the watermelon cv. Sugar Baby (SB) (Intersemillas S. A., Loriguilla, Valencia, Spain)

were used in the experiments. The watermelon cultivar SB was grafted onto the *C. amarus* accessions using the cleft procedure (Lee et al. 2010).

The watermelon SB ungrafted and grafted onto the *C. amarus* accessions were transplanted into 3 L pots filled with sterile sand, and inoculated with the *M. incognita* isolate Agropolis or *M. javanica* isolate MJ05 at initial population densities (Pi) of 0, 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 J2 per cm^3 of sand to assess the relationship between Pi and the nematode densities at the end of the experiment (Pf), and the effect on relative dry shoot biomass. Each treatment was replicated four times. Plants were maintained from May to July (73 d) in a greenhouse. Plants were watered as needed and fertilized with a slow release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with 5TM probes (Decagon devices, Inc.).

At the end of the experiment, plants were removed from the pots. The aboveground part of the plants was dried in an oven at 70 °C for 4 days and weighed. The roots were carefully washed free of soil and weighed. Afterwards, eggs were extracted by blender maceration of all the root system in a 10% commercial bleach solution (40 g/L NaOCl) (Hussey and Barker 1973) and was considered the *Pf*. The maximum multiplication rate (a) was estimated by the slope of the linear regression between Pf and the lowest values of Pi, according to $Pf = aPi$ (Seinhorst 1970). The maximum population density (M) was estimated from the experimental data, and the equilibrium density (E) was estimated by iteration from the regression equation obtained from the relation between Pi and Pf.

2.2.3 Effect of *C. amarus* on *M. incognita* reproduction, disease severity and watermelon yield cultivated in plastic greenhouse

The experiment was conducted in a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain), infested with *M. incognita*. The soil texture was sandy loam with 83.8% sand, 6.7% loam and 9.5% clay; pH 8.7; 1.8% of organic matter (w/w) and 0.5 dS/m electrical conductivity. The experiment consisted in four treatments: SB grafted onto CI64; SB grafted onto CI67, SB grafted onto the *C. lanatus* rootstock cv. Robusta (RO) (Intersemillas S. A., Loriguilla, Valencia, Spain), and the ungrafted SB. Each treatment was replicated 5 times in plots with narrow variation on nematode densities between treatments at transplantation of the first crop in 2017. Crops were carried out from April 10 to August 17 2017 (129 d) and from March 20 to August 7 2018 (140 d). Plots were maintained in black fallow between cropping seasons. Individual plots consisted in a row with 4 plants spaced 0.6 cm. Plots within a row were spaced 1 m. Plants of each treatment were cultivated in the same plot each year to determine the effect on *M. incognita* densities, the disease severity, the crop yield and the durability of the resistance. Soil of each plot was prepared individually to avoid cross contamination. Plants were irrigated as needed trough a drip irrigation system and fertilized with a solution of NPK (15-5-30) at 31 kg/ha, iron chelate and micronutrients at 0.9 kg/ha. Weeds were removed manually before and during the cropping season. Fruit yield were determined 13 and 18 weeks after transplanting the crop in 2017, and at 15 and 20 weeks after transplanting in 2018. Soil temperatures and water content of soil were recorded daily at 1 h intervals with digital probes 5TM (Decagon devices, Inc.) placed at 15 cm depth.

Nematode densities in soil were determined at transplantation (Pi) and at the end of the crop (Pf). Soil samples were taken from each experimental plot and consisted of eight cores taken from the first 30 cm of soil with an auger of diameter 2.5 cm. Soil subsamples were mixed and passed through a 4 mm pore sieve to remove stones. The J2 were extracted from 500 cm^3 of soil using Baermann trays (Whitehead and Hemming 1965) and incubated at 27 ± 2 °C for one week. J2 were collected using

a 25 μm aperture screen, counted, and expressed as J2 per 100 cm^3 of soil. At the end of the crop, roots were carefully uprooted and washed, and the galling index (GI) was evaluated on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots dead (Zeck 1971). After that, the number of eggs per plant were determined extracting them from roots by blender maceration in a 5% commercial bleach solution (40 g/L NaOCl) (Hussey and Barker 1973), as previously stated. The eggs extracted from roots of SB, CI67, and RO at the end of the crop in 2018 were incubated in Baermann trays at 27 ± 2 °C to allow J2 emergence to determine the putative selection for virulence. Reproduction index (RI) was calculated as the percentage of eggs produced in the rootstock with regard to that in the watermelon cultivar. The response of the rootstock was categorized according to the RI as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%), or susceptible (RI ≥ 50%) (Hadisoeganda 1982).

2.2.4 Virulence selection

The nematode inoculum consisted of J2 obtained as previously described. The J2 emerged in the first 24 h were discarded. Afterwards, nematodes were collected daily for 10 days using a 25 μm sieve and stored at 9 °C until inoculation. Seeds of SB, CI67, and RO were germinated according to the method given in Expósito et al. (2018). Seedlings were transplanted to 200 cm^3 pots containing sterile sand and maintained in a growth chamber at 25 ± 2 °C with a 16:8 h (light:dark) photoperiod for a week, and then inoculated with 1 J2 per cm^3 sand. Each treatment was replicated 7 times. Plants were maintained in the growth chamber for 50 days. Plants were watered as needed throughout the experiment and fertilized with a slow-release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with PT100 probes (Campbell Scientific Ltd.).

At the end of the experiments, the roots were carefully washed free of sand. Afterwards, the GI was evaluated, the number of eggs per plant was determined, the RI was calculated, and the level of resistance was categorized following the procedures previously stated.

2.2.5 Statistical analysis

Statistical analyses were performed using R Statistical software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The data were not normally distributed according to the normal Shapiro–Wilk W test. Then, the non-parametric Mann–Whitney U test was used for paired comparisons. The relative yield of grafted or ungrafted watermelon was plotted against the Pi values and submitted to a non-linear regression analysis (proc nlin) of SAS system V9.4 (SAS Institute Inc., Cary, NC) to determine if they fitted the Seinhorst damage function model $y = m + (1 - m)0.95^{Pi/(T-1)}$ when $Pi \geq T$, and $y = 1$ when $Pi < T$, where y is the relative yield, m is the minimum relative yield, and T is the tolerance limit (Seinhorst 1998).

2.3 Results

2.3.1 Relationship between increasing *Pi* of *M. incognita* or *M. javanica* on ungrafted and grafted watermelon and *Pf* and plant biomass

The sand temperature during the experiment ranged from 20.6 °C to 30.6 °C. The maximum multiplication rate (a), the maximum population density (M), and the equilibrium density (E) of the *M. incognita* isolate Agropolis in CI64 and CI67 were 37% and 29%, 72% and 76%, and 69% and 79% lower than in SB, respectively. Regarding the *M. javanica* isolate MJ05, the values of a, M and E in CI64 and CI67 were 99% and 81%, 65% and 76%, and 15% and 33% lower than in SB, respectively (Table 2.1, fig. 2.1). The relationship between Pi and the dry shoot biomass of the ungrafted and grafted

Table 2.1: Maximum multiplication rate (a), maximum population density (M, J2+ eggs per 100 cm^3 of sand) and equilibrium density (E, J2+ eggs per 100 cm^3 of sand) of *M. incognita* and *M. javanica* in ungrafted watermelon cv. Sugar Baby (SB) and grafted in the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67) cultivated in 3 L pots inoculated with 0, 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 J2 per cm^3 of sand (Pi) and maintained in a greenhouse for 73 d from May to July 2016.

Meloidogyne spp.	Rootstock-scion	a	M	E
M. incognita	SB	9.5	3485	3031
	CI64-SB	6.0	969	960
	CI67-SB	6.8	828	684
M. javanica	SB	58.3	5320	1321
	CI64-SB	6.8	1875	1132
	CI67-SB	11.4	12.73	882

watermelon did not fit the Seinhorst's damage model irrespective of the nematode isolate (data not shown).

2.3.2 Effect of *C. amarus* on *M. incognita* reproduction, disease severity and watermelon yield cultivated in plastic greenhouse

The minimum and maximum soil temperatures during the cropping season in 2017 ranged from 13.1 °C to 29.8 °C, and from 13.5 °C to 29.3 °C in 2018. The nematode densities in soil at transplantation ranged from 1 to 53 J2 per 100 cm^3 of soil in 2017 and did not differ ($P < 0.05$) between grafted and ungrafted watermelons, but the galling index (fig. 2.2) and the number of eggs per plant differed ($P < 0.05$) between grafted and ungrafted watermelons at the end of the crop (Table 2.2). All grafted watermelons yielded between 2.9 and 5.6 more kg per plant ($P < 0.05$) than the ungrafted SB. In 2018, nematode densities in soil did not differ ($P < 0.05$) between grafted and ungrafted watermelons either at transplantation nor at the end of the crop, nonetheless higher galling index ($P < 0.05$) and more eggs per plant ($P < 0.05$) were registered in ungrafted than grafted SB. Regarding crop yield, grafted watermelons yielded between 2.0 and 2.7 more kg per plant ($P < 0.05$) than the ungrafted SB. The *C. amarus* accessions performed from highly resistant to resistant ($1\% \leq \text{RI} < 10\%$) to *M. incognita* in both years, and the rootstock RO from moderately resistant ($10\% \leq \text{RI} < 25\%$) in 2017, to slightly resistant ($25\% \leq \text{RI} < 50\%$) in 2018 (Table 2).

2.3.3 Virulence selection

All the *M. incognita* subpopulations infected all the plant germplasm (GI > 1) but none of them reproduced in CI67 and reproduced less ($P < 0.05$) in RO than SB (Table 2.3). The reproduction of the nematode subpopulations in SB did not differ ($P < 0.05$). The rootstock RO performed as resistant to the subpopulation SB but slightly resistant to the subpopulation RO.

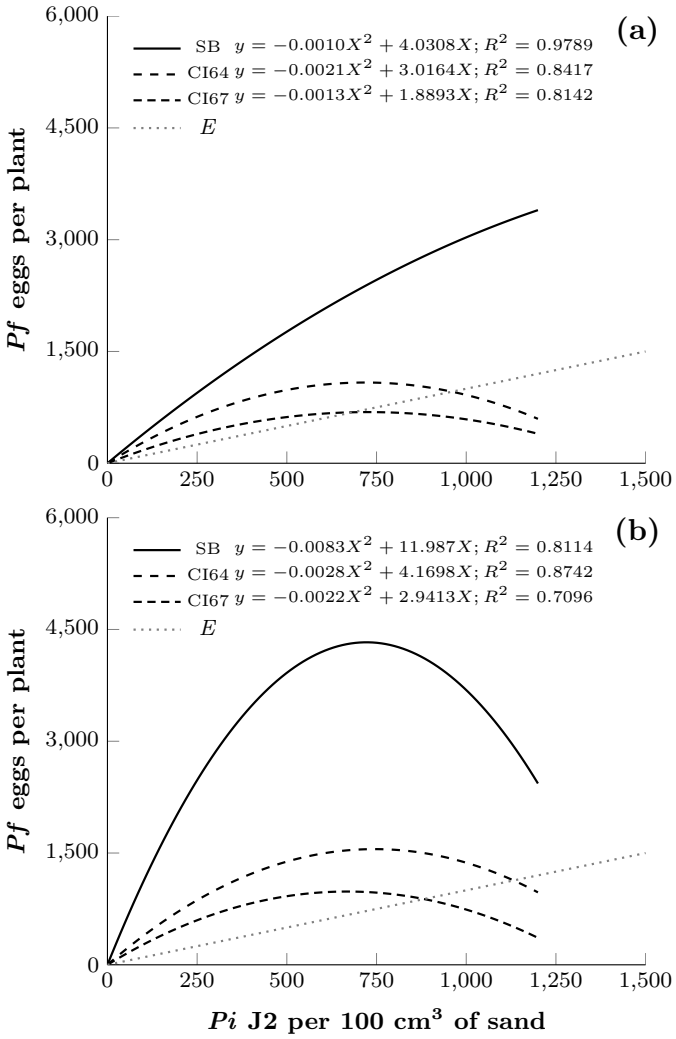


Figure 2.1: Relationship between initial (*Pi*) and final (*Pf*) *M. incognita* (a) and *M. javanica* (b) densities in ungrafted watermelon cv. Sugar Baby (SB) and grafted onto the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67) cultivated in 3 L pots inoculated with 0, 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 J2 per cm^3 of sand (*Pi*) and maintained in a greenhouse for 73 d from May to July 2016.

2.4 Discussion

Both *C. amarus* accessions used in this study have been recently reported as resistant to *M. arenaria*, *M. incognita* and *M. javanica* and also to a virulent *Mi1.2* gene isolate (García-Mendívil et al. 2019), but no information on its response to increasing nematode densities has been previously published. The results of this study show the poorest host status of both *C. amarus* accessions irrespective of the *Meloidogyne* species. The nematode population dynamic parameters in the *C. amarus* accessions were lower than in watermelon, which has been reported as a poor host of *M. javanica* compared to other cucurbits in experiments conducted under the same conditions (Giné et al. 2014; López-Gómez et al. 2014, 2015). The maximum multiplication rate of *M. javanica* in watermelon was 96.7% and 98.3% lower than in zucchini and cucumber, respectively; and the equilibrium density, considered as an indicator of plant tolerance, was 93% lower than in zucchini but 494 times higher than in cucumber. In relation to *M. incognita*, as far we know, there is no information on these population dynamic parameters for watermelon. Interestingly, the results of this study show that watermelon and both *C. amarus* accessions are

Table 2.2: *Meloidogyne incognita* densities in soil at transplantation (*Pi*) and at the end of the crop (*Pf*), galling index (GI), eggs per plant, and reproduction index (RI) of the nematode in the ungrafted (SB) and grafted watermelon cv. Sugar Baby onto the rootstock cv. Robusta (RO), and the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67) cultivated for two consecutive years in the same plot from April to August 2017 (129 days) and March to August (140 days) in plastic greenhouse, and fruit yield per plant.

Rootstock-scion	*Pi* (J2 per 100 cm^3 soil)	*Pf* (J2 per 100 cm^3 soil)	GI[a]	Eggs per plant ($\times 10^2$)	RI (%)[b]	Yield (kg per plant)
first crop (2017):						
SB	14 ± 3	10 ± 5	4.5 ± 0.5	4097 ± 1528		2.7 ± 0.4
RO-SB	24 ± 10	8 ± 4	2.2 ± 0.3*	693 ± 495*	16.9 ± 11.5	5.6 ± 0.7*
CI64-SB	17 ± 8	6 ± 3	2.2 ± 0.3*	40 ± 20*	1.0 ± 0.7	8.3 ± 1.1*
CI67-SB	20 ± 13	4 ± 2	2.5 ± 0.3*	69 ± 24*	1.7 ± 0.6	6.0 ± 0.8*
Second crop (2018):						
SB	11 ± 6	8 ± 4	4.0 ± 0.2	144 ± 44		2.9 ± 0.6
RO-SB	2 ± 2	4 ± 3	1.8 ± 0.3*	52 ± 41*	45.2 ± 37.3	4.9 ± 0.7*
CI64-SB	7 ± 2	2 ± 2	1.1 ± 0.2*	4 ± 2*	2.4 ± 1.9	5.0 ± 0.7*
CI67-SB	9 ± 5	7 ± 2	1.1 ± 0.2*	2 ± 1*	1.1 ± 0.6	5.6 ± 0.9*

Data are the mean ± standard error of 10 replicates. Different letters in the same column indicate significant differences ($P < 0.05$) between germplasm according to the Kruskal–Wallis test.

[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck 1971).

[b]Reproduction index = 100 × (number of eggs per plant produced in the CI accessions or Robusta / mean number of eggs per plant produced in the susceptible cv. Sugar Baby).

less suitable hosts for *M. incognita* than for *M. javanica* according to the lower values of maximum multiplication rate and maximum population density. However, watermelon, conversely to both *C. amarus* accessions, would tolerate better *M. incognita* than *M. javanica* according to the *E* values. In our experiments, the *C. amarus* accessions along with the watermelon cultivar tolerates both *M. incognita* and *M. javanica* since no differences in dry top weight biomass were found when submitted to *Pi* levels from 0 to 1200 J2 per 100 cm^3 of soil. Lopez-Gomez et al. (2016) also did not find any differences at *Pi* levels from 0 to 1000 J2 per 100 cm^3 of soil in pot experiments, but they did at *Pi* levels from 0 to 51200 J2 per 100 cm^3 of soil (López-Gómez et al. 2014).

The resistance level of both *C. amarus* accessions was maintained after being cultivated for two consecutive growing seasons in the same plots under plastic greenhouse conditions. In addition, no selection for virulence to *C. amarus* was found according to the results of the experiment conducted with the nematode subpopulations obtained from roots of each germplasm at the end of the second crop. These results are in agreement with that reported by Thies et al. (2010) who found that the resistance of some *C. amarus* assessed in field conditions was maintained after two consecutive years of cultivation. The resistance of *C. amarus* to RKN has been associated with the root fibrosity (Thies and Levi 2003, 2007; Thies et al. 2015c, 2016). In this study, the level of root fibrosity was not assessed but visual comparison between the root systems of all the assessed germplasms suggest that those of the *C. amarus* accessions were more fibrous than that of watermelon, as has been shown in fig. 2.2. In fact, Thies et al., (2010) proposed the assessment of the percentage of fibrous roots as an additional trait for identifying RKN resistance in *C. amarus*.

Regarding watermelon yield, grafting increased between 2 and 5.6 kg per plant compared to the ungrafted watermelon. Previous studies reported that grafting water-

Table 2.3: Galling index, number of eggs per plant, and reproduction index of *M. incognita* subpopulations in the watermelon cv. Sugar Baby (SB), the rootstock cv. Robusta (RO), and the *C. amarus* accession BGV0005167 (CI67) 50 days after cultivation in 200 cm^3 pots inoculated with 1 J2 per cm^3 of soil. The *M. incognita* were obtained from infected roots of the ungrafted SB (SP-SB), grafted onto RO (SP-RO) and onto CI67 (SP-CI67) after cultivation for two consecutive cropping seasons (2017 and 2018) in the same plots in a plastic greenhouse.

Plant	Galling index[a]			Eggs per plant ($\times 10^2$)			Reproduction index (%)[b]		
host	SP-SB	SP-RO	SP-CI67	SP-SB	SP-RO	SP-CI67	SP-SB	SP-RO	ISP-CI67
SB	2.8 ± 0.4	3.0 ± 0.5	3.0 ± 0.4	11.3 ± 7.1	4.4 ± 1.5	3.1 ± 1.4			
RO	2.0 ± 0.4	1.5 ± 0.5	Na	0.9 ± 0.6*	1.2 ± 1.1*	Na	8.2	27.6	Na
CI67	1.4 ± 0.6	0.7 ± 0.2*	1.0 ± 0.4*	0 ± 0*	0 ± 0*	0 ± 0*	0	0	0

Data are mean ± standard error of 7 replicates. Values followed by * in the same column show significant differences ($P < 0.05$) between each germplasm and the watermelon SB, and values in the same row and parameter followed by + indicate significant differences ($P < 0.05$) respect the SP-SB nematode subpopulation according to the non-parametric Mann–Whitney U test; Na: Not assessed.
[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck 1971).
[b]Reproduction index = 100 × (number of eggs per plant in a given rootstock / number of eggs per plant on the watermelon cv. Sugar Baby).

melon onto *C. amarus* lines yielded between 1.3 and 1.9 more times than the non-grafted cultivated in heavily infested soils (Thies et al. 2015a, 2015b, 2015c), and also when grafted onto cv. Carolina Strongback and cultivated in soils infested with *Fusarium oxysporum* f. sp. *niveum* (Fon) race 2 and *M. incognita* but not in soils infested with *M. incognita* alone (Keinath et al. 2019).

Among the *C. amarus* accessions assessed in this study, the CI67 has been shown compatible with watermelon without affecting fruit quality (Fredes et al. 2017; García-Mendívil et al. 2019). Then, this accession might be an effective alternative to other watermelon rootstocks. In addition, it might be an additional resistant genetic source to be included in rotation schemes for managing the most widespread tropical *Meloidogyne* species, and could affect the level of virulence for a given *R*-gene if selection occurs as reported by Expósito et al. (2019) including *Cucumis metuliferus* in the rotation schema. In addition, the reduced ability of the nematode to reproduce in CI67 could leave low nematode densities at transplantation of the following crop, which will suffer less damage and crop yield losses (Ornat et al. 1997; Talavera et al. 2009; Giné et al. 2017; Expósito et al. 2019).

2.5 Conclusion

The results of this study highlight the poorest host status of the CI64 and CI67 accessions to *M. incognita* and *M. javanica*; its tolerance to both *Meloidogyne* species; the stability of the resistance; and its productivity when cropped in infested soils.

Acknowledgments

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R and AGL2017-89785-R, and CONACYT for providing PhD funding to H. A. García-Mendívil. Thank are also given to María Belén Picó and Carmina Gisbert for providing BGV0005164 and BVG0005167 seeds, and to Ariadna Giné, Sergi García, Alejandro Expósito, Anna Sanz, and Miquel Masip for technical assistance.

Figure 2.2: Root system of the watermelon cv. Sugar Baby (A), the *C. lanatus* rootstock cv. Robusta (B), the *C. amarus* accessions BGV0005164 (C) and BGV0005167 (D), cultivated in plastic greenhouse infested with *M. incognita*.

References

Expósito, A., S. García, A. Giné, N. Escudero, and F. J. Sorribas. 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato–melon rotation sequence. *Pest Management Science* 75, no. 7 (): 1902–1910. ISSN: 1526-498X. doi:10.1002/ps.5297. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.5297.

Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, M. B. Picó, et al. 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67, no. 5 (): 1161–1167. ISSN: 00320862. doi:10.1111/ppa.12815. http://doi.wiley.com/10.1111/ppa.12815.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Statistics Division. Visited on 09/04/2019. http://www.fao.org/faostat.

Fredes, A., S. Roselló, J. Beltrán, J. Cebolla-Cornejo, A. Pérez-de-Castro, C. Gisbert, et al. 2017. Fruit quality assessment of watermelons grafted onto citron melon rootstock. *Journal of the Science of Food and Agriculture* 97, no. 5 (): 1646–1655. ISSN: 00225142. doi:10.1002/jsfa.7915. http://doi.wiley.com/10.1002/jsfa.7915.

García-Mendívil, H. A., M. Munera, A. Giné, N. Escudero, M. B. Picó, C. Gisbert, et al. 2019. Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. *Crop Protection* 119 (): 208–213. ISSN: 02612194. doi:10.1016/j.cropro.2019.02.005. https://linkinghub.elsevier.com/retrieve/pii/S0261219419300432.

Giné, A., C. González, L. Serrano, and F. J. Sorribas. 2017. Population dynamics of *Meloidogyne incognita* on cucumber grafted onto the Cucurbita hybrid RS841 or ungrafted and yield losses under protected cultivation. *European Journal of Plant Pathology* 148, no. 4 (): 795–805. ISSN: 0929-1873. doi:10.1007/s10658-016-1135-z. http://link.springer.com/10.1007/s10658-016-1135-z.

Giné, A., M. López-Gómez, M. D. Vela, C. Ornat, M. Talavera, S. Verdejo-Lucas, et al. 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology* 63, no. 6 (): 1446–1453. ISSN: 00320862. doi:10.1111/ppa.12217. http://doi.wiley.com/10.1111/ppa.12217.

Hadisoeganda, W. W. 1982. Resistance of Tomato, Bean, Southern Pea, and Garden Pea Cultivars to Root-Knot Nematodes Based on Host Suitability. *Plant Disease* 66 (1): 145. ISSN: 0191-2917. doi:10.1094/PD-66-145. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1982Abstracts/PD%7B%5C_%7D66%7B%5C_%7D145.htm.

Hallmann, J., and B. H. Meressa. 2018. Nematode parasites of vegetables. In *Plant parasitic nematodes in subtropical and tropical agriculture*, ed. by R. Sikora, D. Coyne, J. Hallman, and P. Timper. Wallingford: CABI. doi:10.1079/9781786391247.0346.

Huitrón, M. V., M. Diaz, F. Diánez, and F. Camacho. 2007. The effect of various rootstocks on triploid watermelon yield and quality. *Journal of Food, Agriculture and Environment*. ISSN: 14590263. https://pubag.nal.usda.gov/catalog/723527.

Hussey, R. S., and K. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025–1028.

Keinath, A. P., W. P. Wechter, W. B. Rutter, and P. A. Agudelo. 2019. Cucurbit Rootstocks Resistant to *Fusarium oxysporum* f. sp. *niveum* Remain Resistant When Coinfected by *Meloidogyne incognita* in the Field. *Plant Disease* 103, no. 6 (): 1383–1390. ISSN: 0191-2917. doi:10.1094/PDIS-10-18-1869-RE. https://apsjournals.apsnet.org/doi/10.1094/PDIS-10-18-1869-RE.

Kemble, J. M., I. M. Meadows, K. M. Jennings, and J. F. Walgenbach. 2019. *Southeastern U.S. Vegetable Crop Handbook*. https://www.growingproduce.com/southeasternvegetablecrophandbook/.

Lee, J. M., C. Kubota, S. J. Tsao, Z. Bie, P. H. Echevarria, L. Morra, et al. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. doi:10.1016/j.scienta.2010.08.003.

Levi, A., R. Jarret, S. Kousik, W. Patrick Wechter, P. Nimmakayala, and U. K. Reddy. 2017. Genetic Resources of Watermelon. In *Plant Genetics and Genomics: Crops and Models*, 87–110. Springer International. doi:10.1007/7397_2016_34. http://link.springer.com/10.1007/7397%7B%5C_%7D2016%7B%5C_%7D34.

López-Gómez, M., E. Flor-Peregrín, M. Talavera, F. J. Sorribas, and S. Verdejo-Lucas. 2015. Population dynamics of *Meloidogyne javanica* and its relationship with the leaf chlorophyll content in zucchini. *Crop Protection* 70 (): 8–14. ISSN: 02612194. doi:10.1016/j.cropro.2014.12.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219414003871.

López-Gómez, M., A. Giné, M. D. Vela, C. Ornat, F. J. Sorribas, M. Talavera, et al. 2014. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. *Annals of Applied Biology* 165, no. 3 (): 466–473. ISSN: 00034746. doi:10.1111/aab.12154. http://doi.wiley.com/10.1111/aab.12154.

López-Gómez, M., M. Talavera, and S. Verdejo-Lucas. 2016. Differential reproduction of *Meloidogyne incognita* and *M. javanica* in watermelon cultivars and cucurbit rootstocks. *Plant Pathology* 65, no. 1 (): 145–153. ISSN: 13653059. doi:10.1111/ppa.12394. http://doi.wiley.com/10.1111/ppa.12394.

Miguel, A., J. V. Maroto, A. San Bautista, C. Baixauli, V. Cebolla, B. Pascual, et al. 2004. The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. *Scientia Horticulturae* 103, no. 1 (): 9–17. ISSN: 03044238. doi:10.1016/j.scienta.2004.04.007. https://linkinghub.elsevier.com/retrieve/pii/S030442380400086X.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 1997. Effect of the previous crop on population densities of *Meloidogyne javanica* and yield of cucumber. *Nematropica*. ISSN: 0099-5444.

Seinhorst, J. W. 1970. Dynamics of Populations of Plant Parasitic Nematodes. *Annual Review of Phytopathology* 8, no. 1 (): 131–156. ISSN: 0066-4286. doi:10.1146/annurev.py.08.090170.001023. http://www.annualreviews.org/doi/10.1146/annurev.py.08.090170.001023.

— . 1998. The common relation between population density and plant weight in pot and microplot experiments with various nematode plant combinations. *Fundamental and Applied Nematology* 21:459–468. ISSN: 1164-5571. http://www.documentation.ird.fr/hor/fdi:010017629.

Talavera, M., S. Verdejo-Lucas, C. Ornat, J. Torres, M. D. Vela, F. J. Macias, et al. 2009. Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses. *Crop Protection* 28, no. 8 (): 662–667. ISSN: 02612194. doi:10.1016/j.cropro.2009.03.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219409000763.

Thies, J. A., J. J. Ariss, R. L. Hassell, S. Buckner, and A. Levi. 2015a. Accessions of *Citrullus lanatus* var. *citroides* Are Valuable Rootstocks for Grafted Watermelon in Fields Infested with Root-knot Nematodes. *HortScience* 50, no. 1 (): 4–8. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.4. https://journals.ashs.org/view/journals/hortsci/50/1/article-p4.xml.

Thies, J. A., J. J. Ariss, R. L. Hassell, S. Olson, C. S. Kousik, and A. Levi. 2010. Grafting for Management of Southern Root-Knot Nematode, *Meloidogyne incognita*, in Watermelon. *Plant Disease* 94, no. 10 (): 1195–1199. ISSN: 0191-2917. doi:10.1094/PDIS-09-09-0640. http://apsjournals.apsnet.org/doi/10.1094/PDIS-09-09-0640.

Thies, J. A., J. J. Ariss, C. S. Kousik, R. L. Hassell, and A. Levi. 2016. Resistance to Southern Root-knot Nematode (*Meloidogyne incognita*) in Wild Watermelon (*Citrullus lanatus* var. *citroides*). *Journal of Nematology* 48 (1): 14–19. ISSN: 0022-300X. doi:10.21307/jofnem-2017-004. https://www.exeley.com/journal%7B%5C_%7Dof%7B%5C_%7Dnematology/doi/10.21307/jofnem-2017-004.

Thies, J. A., S. Buckner, M. Horry, R. Hassell, and A. Levi. 2015b. Influence of *Citrullus lanatus* var. *citroides* Rootstocks and Their F1 Hybrids on Yield and Response to Root-knot Nematode, *Meloidogyne incognita*, in Grafted Watermelon. *HortScience* 50, no. 1 (): 9–12. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.9. https://journals.ashs.org/view/journals/hortsci/50/1/article-p9.xml.

Thies, J. A., and A. Levi. 2003. Resistance of Watermelon Germplasm to the Peanut Root-knot Nematode. *HortScience* 38, no. 7 (): 1417–1421. ISSN: 0018-5345. doi:10.21273/HORTSCI.38.7.1417. https://journals.ashs.org/view/journals/hortsci/38/7/article-p1417.xml.

— . 2007. Characterization of Watermelon (*Citrullus lanatus* var. *citroides*) Germplasm for Resistance to Root-knot Nematodes. *HortScience* 42, no. 7 (): 1530–1533. ISSN: 0018-5345. doi:10.21273/HORTSCI.42.7.1530. https://journals.ashs.org/view/journals/hortsci/42/7/article-p1530.xml.

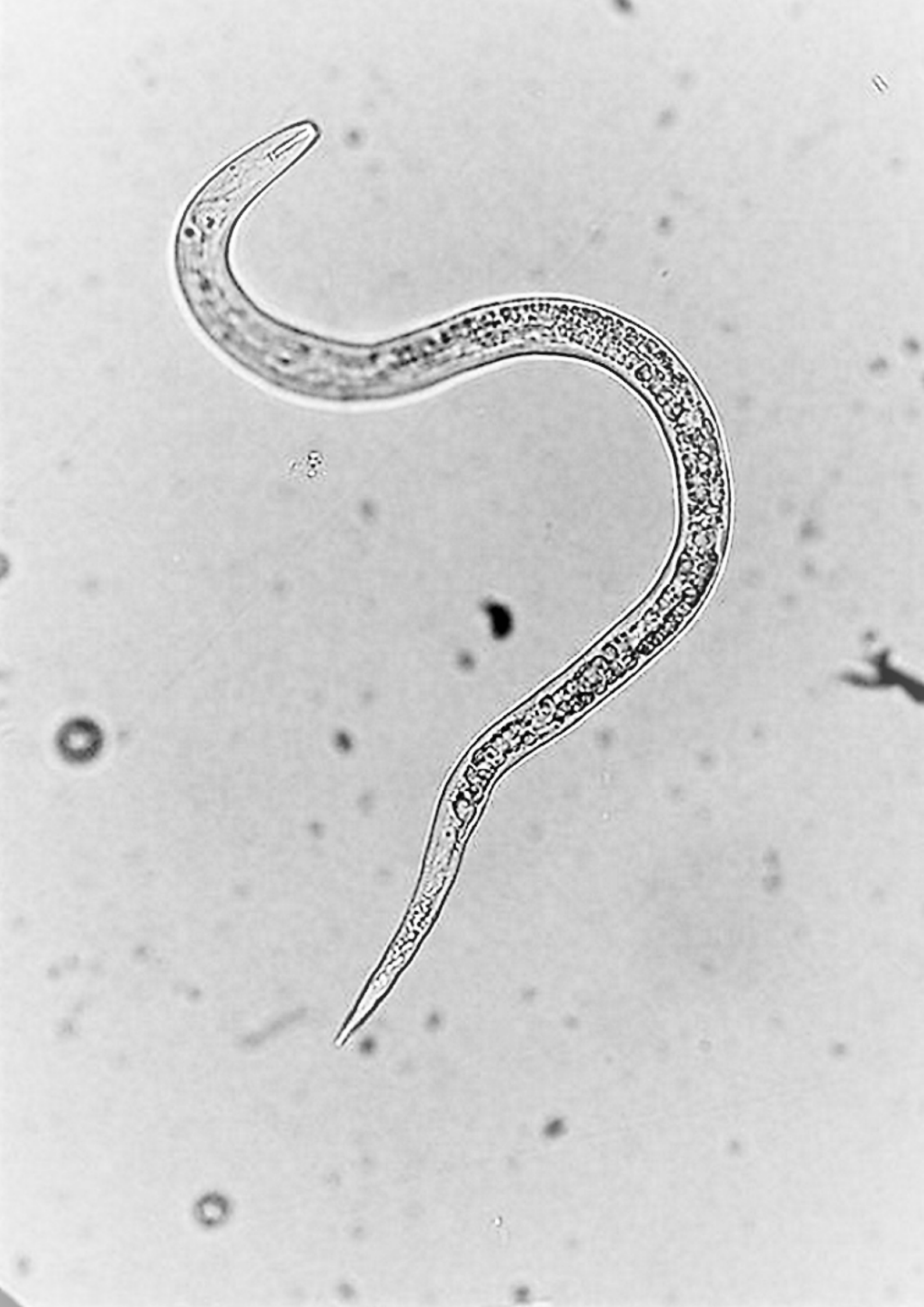
Thies, J. A., A. Levi, J. J. Ariss, and R. L. Hassell. 2015c. RKVL-318, a Root-knot Nematode-resistant Watermelon Line as Rootstock for Grafted Watermelon. *HortScience* 50, no. 1 (): 141–142. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.141. https://journals.ashs.org/view/journals/hortsci/50/1/article-p141.xml.

Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, no. 1 (): 25–38. ISSN: 0003-4746. doi:10.1111/j.1744-7348.

1965.tb07864.x. http://doi.wiley.com/10.1111/j.1744-7348.1965.tb07864.x.

Zeck, W. M. 1971. Rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschutz nachrichten*, no. 24: 141–144. ISSN: 0079-1342.

Zijlstra, C., D. T. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2 (8): 847–853. ISSN: 1388-5545. doi:10.1163/156854100750112798. https://brill.com/view/journals/nemy/2/8/article-p847%7B%5C_%7D3.xml.



Chapter 3

Host suitability of *Solanum torvum* cultivars to *Meloidogyne incognita* and *M. javanica* and population dynamics

Host suitability of *Solanum torvum* cultivars to *Meloidogyne incognita* and *M. javanica* and population dynamics

Helio A.García-Mendívil, Nuria Escudero and Francisco J. Sorribas

2019. *Plant Pathology*, ppa.13036. https://doi.org/10.1111/ppa.13036

Abstract

Several experiments were carried out to assess the performance of commercial *Solanum torvum* cultivars against the root knot nematodes *Meloidogyne incognita* and *M. javanica* in Spain. The response of *S. torvum* rootstock cultivars Brutus, Espina, Salutamu and Torpedo against *M. incognita* and *Mi1.2* (a)virulent *M. javanica* isolates was determined in pot experiments, and of 'Brutus' to an *N*-virulent isolate of *M. incognita*, compared with that of the eggplant *S. melongena* 'Cristal'. The relationship between the initial and final population densities of *M. javanica* on ungrafted and grafted 'Cristal' onto the *S. torvum* 'Brutus' was assessed, together with the effect on dry shoot biomass. finally, the population growth rate and the resistance level of the four *S. torvum* cultivars against *M. incognita* was assessed under plastic greenhouse conditions in two cropping seasons. All *S. torvum* rootstocks responded as resistant to the *M. incognita* isolates and from highly resistant to susceptible against *M. javanica* isolates. The maximum multiplication rates of *M. javanica* on the ungrafted or grafted eggplant were 270 and 49, respectively, and the equilibrium densities were 1318 and 2056 eggs and J2 per 100 cm^3 soil, respectively. The tolerance of the ungrafted eggplant was 10.9 J2 per 100 cm^3 soil, and the minimum relative dry shoot biomass was 0.76. The population growth rate of *M. incognita* on eggplant cv. Cristal differed from that of the *S. torvum* cultivars in both cropping seasons. These results suggest that *S. torvum* is a valuable rootstock for managing the two *Meloidogyne* species irrespective of the (a)virulence status.

3.1 Introduction

Root knot nematodes (RKN), *Meloidogyne* spp., are the most damaging plant-parasitic nematodes worldwide. This genus comprises more than 100 species, but four of them are responsible for the majority of crop yield losses: the tropical species *M. arenaria*, *M. incognita* and *M. javanica*, and the temperate species *M. hapla* (Jones et al. 2013). RKN can cause severe damage in solanaceous crops, depending on the plant germplasm, the nematode soil density at transplanting, the (a)virulence status of the population, and the environmental conditions. Estimations of yield losses caused by RKN in several crops have been summarized (Greco and Di Vito 2009). Regarding fruiting solanaceous crops, maximum yield losses of 94, 95 and 100% have been reported for pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), and tomato (*S. lycopersicum*) in microplot experiments, respectively. Although RKN control has broadly been done using soil fumigants and nonfumigant nematicides, current regulations, including the European Directive 2009/128/EC and the US Clean Air Act (US Environmental Protection Agency, 2012), are encouraging the research and development of environment-friendly alternative management strategies. The use of plant resistance is an effective and economically profitable approach for RKN management (Sorribas et al. 2005). The commercial RKN resistant-tolerant germplasm reduces both the infectivity and reproduction

of the nematode, and reduces crop yield losses (Talavera et al. 2009; Giné and Sorribas 2017; Expósito et al. 2019). RKN resistant germplasm comprises both cultivars and rootstocks. Grafting is a useful option when no commercial RKN resistant cultivars are available or when other resistance/tolerance genes are needed for complementing the management of other economically important soilborne diseases and/or abiotic stresses but have not been introgressed into commercial cultivars (Kumar et al. 2018). Despite these advantages, plant resistance must be used properly to avoid selection for virulence by the repeated cultivation of plant germplasm carrying the same resistance (R) gene (Verdejo-Lucas et al. 2009; Giné and Sorribas 2017); and also to reduce the expression of plant resistance due to high soil temperatures (Araujo et al. 1982).

Eggplant is one of the most cultivated solanaceous crops with an estimated worldwide production of *c.* 52 million tonnes in 1.8 million ha (FAOSTAT 2017). This plant species is affected by some soilborne pathogens including RKN. To control them, eggplant has been mostly grafted onto resistant tomato or tomato interspecific hybrids (*S. lycopersicum* × *S. habrochaites*) (Daunay 2008). However, the continuous use of tomato rootstocks induces the selection for virulence (Verdejo-Lucas et al. 2009; Expósito et al. 2019). Consequently, other resistant germplasms have been assessed, including *S. melongena* lines, interspecific hybrids of *S. integrifolium* × *S. melongena*,and wild related species such as *S. integrifolium*, *S. sisymbriifolium* and *S. torvum* (Daunay 2008). Among these wild species, only *S. torvum* is currently commercially available to be employed as a rootstock for eggplant cultivation worldwide (Uehara et al. 2017b; Öçal et al. 2018).

Some *S. torvum* cultivars are resistant to a wide range of soilborne diseases, including fungal and bacterial wilt diseases (Daunay 2008). Regarding RKN, *S. torvum* accessions and cultivars have been consistently described as resistant to *M. incognita* (Ali et al. 1992; Rahman et al. 2002; Dhivya et al. 2014), *M. luci* (Öçal et al. 2018), and some populations of *M. arenaria* and *M. javanica* (Tzortzakakis et al. 2006; Uehara et al. 2017b; Öçal et al. 2018); but are described as susceptible to *M. hapla* (Öçal et al. 2018). As far as the authors know, there is no information about the performance of commercial *S. torvum* cultivars against *M. incognita* and *M. javanica* populations from Spain, the most frequent RKN species in vegetable growing areas. Some are known to be virulent to the *Mi1.2* or the *N* genes in tomato and pepper, respectively (Ornat et al. 2001; Robertson et al. 2006; Verdejo-Lucas et al. 2012).

The design of effective and profitable management strategies must consider the host status, the resistance level and the tolerance of the plant species and germplasm to RKN species and local populations. The host status is estimated by three parameters obtained from the relationship between the nematode population density at transplanting (initial population density, Pi) and at the end of the crop (final population density, Pf): (i) the maximum multiplication rate in the absence of limiting factors (a); (ii) the maximum population density achieved by the nematode under specific conditions (M); and (iii) the equilibrium density at which the plant can supply enough nutrients to a given Pi to maintain this density at the end of the crop (E, $Pf = Pi$; $Pf/Pi = 1$) (Seinhorst 1970). The values of these parameters are higher in susceptible than in resistant or poor hosts. Another useful indicator is the population growth rate, estimated by the relationship between the Pi and the multiplication rate (Pf/Pi), which allows comparison of nematode population dynamics between plant species or germplasms, as well as the efficacy of control methods (Talavera et al. 2009; Giné and Sorribas 2017; Expósito et al. 2019). The resistance level of a given plant germplasm is categorized according to the reproduction index (RI), expressed as the percentage of RKN reproduction in a plant germplasm with regard to that in a susceptible standard plant host (Hadisoeganda 1982). Plant tolerance (T), the maximum Pi at which no

yield losses is recorded, is estimated by the relationship between increasing *Pi* and the relative plant biomass or crop yield. This parameter, along with the minimum relative yield (m), can be estimated by the Seinhorst's damage function model (Seinhorst 1998). Thus, several experiments were conducted to characterize the host suitability of four commercial *S. torvum* rootstocks against several *M. incognita* and *M. javanica* isolates, including virulent isolates to the *Mi1.2* or *N* genes; to estimate the population dynamic of *M. javanica* on ungrafted and grafted eggplant onto *S. torvum* 'Brutus' and also the plant tolerance and the minimum relative dry shoot biomass; and to determine the effect of increasing *Pi* and cropping season on the *M. incognita* population growth rate and the level of resistance.

3.2 Materials and methods

3.2.1 Plant material

The four *S. torvum* rootstocks cultivars Brutus (TB), Espina (TE), Salutamu (TS) and Torpedo (TT), and the eggplant cv. Cristal (MC) were used in the experiments. The main characteristics of these cultivars are presented in Table 3.1. Seeds of the *S. torvum* cultivars were pretreated with a KNO_3 solution to improve germination (Ranil et al. 2015). Afterwards, seeds were transferred to vermiculite-filled trays and incubated in a growth chamber at 25 ± 2 °C and a 16:8 h light:dark photoperiod for 4 weeks. Seedlings were transplanted singly to pots containing sterilized river sand.

3.2.2 Nematode inoculum

Six *M. incognita* and eight *M. javanica* isolates were used in the experiments (Table 3.2). All RKN isolates were maintained on the susceptible tomato cv. Durinta (Seminis Seeds). The *M. javanica* MJLg and *M. incognita* MILgN field isolates were provided by M. López-Gómez (Rijk Zwaan Iberica). These isolates were obtained from fields in which resistant tomato or pepper cultivars were the main crops, and in which a possible selection for virulence might have occurred. Virulence status of the MJLg isolate to the *Mi1.2* gene in tomato, and of the MILgN isolate to the *N* gene in pepper was confirmed 55 days after cultivation of the resistant tomato cv. Monika and the susceptible cv. Durinta, and the resistant pepper cv. Solfeo and the susceptible cv. Compás in 200 cm^3 pots inoculated with 1 second-juvenile (J2)/cm^3 of sand and maintained in a growth chamber at 25 ± 2 °C. The number of eggs produced by the MJLg and MILgN isolates in the resistant tomato and pepper were a 98.3% and 89.3% of those produced in the susceptible tomato and pepper cultivars, respectively (Table 3.3, Results section). All the *Meloidogyne* isolates were identified by SCAR-PCR markers (Zijlstra et al. 2000)

The inoculum used in the pot experiments consisted in J2 emerged from eggs extracted from infected roots by maceration of roots using a 5% commercial bleach solution (40 g/L NaOCl) during 10 min (Hussey and Barker 1973). The J2 emerged during the first 24 h were discarded. After that, J2 were recovered every two days and maintained at 9°C for 8 days until the pot experiments were carried out.

3.2.3 Host suitability

Three pot experiments were carried out to evaluate the response of the four *S. torvum* rootstocks against *Meloidogyne* spp. In the first experiment, the response of the *S. torvum* rootstocks was assessed against five avirulent isolates of *M. incognita* and six of *M. javanica*. Plants were transplanted to 1 L pots and maintained in a greenhouse for 40 days. In the second experiment, the *S. torvum* rootstocks were assessed against the *M. javanica Mi1.2*-virulent isolate MJLg in 200 cm^3 pots and maintained in a growth chamber at 25 ± 2 °C with a 16:8h (light:dark) photoperiod for 55 days. In the third experiment, the response of the selected *S. torvum* rootstock TB was assessed against the *M. javanica Mi1.2*-virulent isolate MJ27 and the *N*-virulent *M. incognita* isolate MILgN in 500 cm^3 pots and maintained for 49 days in a greenhouse. The rootstock cv. Brutus

was selected because showed the most stable response against the most RKN isolates assessed. The eggplant MC was included in all experiment as susceptible control for comparison. Each plant–RKN isolate combination was replicated seven times and the experiments conducted twice, with two weeks apart between each repetition.

All experiments were conducted following the same procedure. Plants were inoculated with 1 J2/cm^3 of sand. Plants were watered as needed throughout the experiment and fertilized with a slow release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with PT100 probes (Campbell Scientific Ltd.).

At the end of the experiments, roots were carefully removed from the soil and washed. The final population density (Pf) was determined extracting the eggs by maceration of roots in a 10% commercial bleach solution (40 g/L NaOCl) for 10 min (Hussey and Barker 1973) and final counting. Reproduction index (RI) was calculated as the percentage of eggs produced in the rootstock cultivar with regard to that in the eggplant cultivar. The response of the plant host was categorized according to the RI as highly resistant (RI $<$ 1%), resistant (1% $\leq$ RI $<$ 10%), moderately resistant (10% $\leq$ RI $<$ 25%), slightly resistant (25% $\leq$ RI $<$ 50%) or susceptible (RI $\geq$ 50%) (Hadisoeganda 1982).

3.2.4 Population dynamics of *M. javanica* on ungrafted and grafted eggplant

The eggplant MC ungrafted and grafted onto the *S. torvum* rootstock cv. TB were transplanted into 500 cm^3 pots filled with sterile sand, and inoculated with the *M. javanica* isolate MJ27 at 0, 0.1, 0.2, 0.4, 0.8, 1.5, 3, 6 and 12 J2/cm^3 of sand (initial population density, Pi) to assess the relationship between Pi and Pf, and the effect on relative dry shoot biomass. Each treatment was replicated four times. Plants were maintained in a greenhouse for 55 days, from June to August, 2017. Plants were watered as needed and fertilized with Osmocote Plus. Soil temperatures were also recorded daily at 30 min intervals with 5TM probes.

At the end of the experiment, plants were uprooted, the roots were carefully washed free of sand and weighed. The aboveground part of the plant (leaves, stems, flowers and fruits) were dried in an oven at 70 °C for 4 days and weighed. The Pf was determined as described previously. The maximum multiplication rate (a) was estimated by the slope of the linear regression between Pf and the lowest values of Pi, according to $Pf = aPi$ (Seinhorst 1970). The maximum population density (M) was estimated from the experimental data, and the equilibrium density (E) was estimated by iteration from the regression equation obtained from the relation between Pi and Pf.

3.2.5 Effect of increasing *Pi* and seasonal cultivation on *M. incognita*

Two experiments were conducted in a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain), infested with *M. incognita*. The first experiment was conducted from May 10 to June 27, 2016 (48 days), and the second from October 4, 2016 to January 31, 2017 (119 days). The *S. torvum* rootstocks TB, TE, TS and TT, and the susceptible eggplant MC were cultivated in 10 and 15 (2.5 m long) plots in the first and second experiments, respectively. In each single plot, one plant of each germplasm was transplanted with a space of 0.5 m, in such a way that every germplasm was an equal number of times nearby to both the edge of the plots and the susceptible MC. Plants were irrigated as needed through a drip irrigation system, and weekly fertilized with a solution consisting of NPK (15-5-30) at 31 kg/ha and iron chelate and micronutrients at 0.9 kg/ha. The temperature was recorded at 30 min interval with temperature probes 5TM (Decagon devices, Inc.) at a depth of 15 cm.

Nematode population density was determined at transplantation (Pi). Soil samples consisted of eight cores taken from the first

30 cm of soil with an auger of diameter 2.5 cm. Soil subsamples were mixed and sieved (4 mm) to remove stones. For each experimental plot, J2 were extracted from 500 cm^3 of soil using Baermann trays (Whitehead and Hemming 1965) and incubated at 27 ± 2 °C for a week. J2 were counted and expressed as J2 per 250 cm^3 of soil. At the end of the experiments, roots were carefully removed from the soil, washed, and weighed, and the galling index (GI) was estimated in a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots dead (Zeck 1971). The Pf was determined from all the root system, the RI was calculated and the resistance level categorized as described previously. The maximum multiplication rate (a) and the maximum population density (M) were estimated as described previously, and the equilibrium density (E) from the linear regression equation obtained from the relationship between $\log_{10}$ Pi and $\log_{10}$ Pf/Pi.

3.2.6 Statistical analysis

Statistical analysis was performed using R Statistical Software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The data from the host suitability experiments were not normally distributed according to the normal Shapiro–Wilk W test. Then, data were submitted to the nonparametric Mann–Whitney U test to compare between repetitions of the same experiment. When no differences were found ($P < 0.05$), data were pooled and considered as replications of the same experiment. Comparisons between plant germplasm per each RKN isolate as well as between RKN isolates per each plant germplasm within each experiment were done by the Mann–Whitney U test (two groups) or the Kruskal–Wallis non parametric test (more than two groups). When significant ($P < 0.05$), medians were subsequently separated using pairwise multiple comparisons by the Dunn test ($P < 0.05$). The nonlinear procedure (proc nlin) of SAS system V9.4 (SAS Institute Inc., Cary, NC) was used to determine the compliance of the data to the Seinhorst damage function model (Seinhorst 1998); $y = m + (1 - m)0.95^{Pi/(T-1)}$ when $Pi \geq T$, and $y = 1$ when $Pi < T$, where y is the relative dry shoot biomass, m is the minimum relative dry shoot biomass, and T is the tolerance limit. The values of Pi and Pf/Pi were transformed to $\log_{10}$ to linearize the relationship between Pi and Pi/Pf and the regression of each rootstock cultivar was compared to that of the eggplant MC. When the Pi values of different experimental plots per seasonal cultivation were closer the mean was calculated (Schomaker and Been 2013) and used for the regression analysis between Pi and Pf/Pi.

3.3 Results

3.3.1 Host suitability

The minimal, maximal and mean sand temperatures recorded in the greenhouse conditions experiments were 23.3°C, 30.0 °C and 27.4 °C, respectively, in experiment 1; and 17.8 °C, 25.6°C and 22.8 °C in experiment 3. All RKN isolates produced less ($P < 0.05$) number of eggs in the *S. torvum* rootstocks cultivars than in the eggplant cv. MC, irrespective of the nematode species and virulence status. The response of all *S. torvum* rootstocks ranged between highly resistant (RI < 1%) (3 out of 21 combinations) and resistant (10% ≤ RI < 10%) (18 out of 21 combinations) to the *M. incognita* isolates. But they responded mainly as resistant and moderately resistant (15 and 10 out 29 combinations, respectively) to the *M. javanica* isolates (Table 3.4).

3.3.2 Population dynamics of *M. javanica* on ungrafted and grafted eggplant

The minimum, maximum and mean sand temperatures recorded were 23.9, 27.8 and 26.2 °C, respectively. The maximum multiplication rate (a), maximum density (M) and equilibrium density (E) of the *M. javanica* isolate MJ27 on the ungrafted eggplant MC were 270, 30 906 eggs+J2 per 100 cm^3, and 1318 eggs+J2 per 100 cm^3, respectively. In the grafted MC onto TB the values

of a, M and E were 49, 4223 eggs+J2 per 100 cm^3, and 2056 eggs+J2 per cm^3, respectively (fig. 3.1). The relationship between Pi and dry shoot weight biomass for the ungrafted eggplant fitted to the Seinhorst damage model ($P < 0.0001$, $R^2 = 0.998$), but that of the grafted eggplant did not. The tolerance (T) of the ungrafted eggplant was 10.9 J2 per 100 cm^3 soil, and the minimum dry shoot biomass (m) was 0.76 (fig. 3.2).

3.3.3 Effect of increasing *Pi* and seasonal cultivation on *M. incognita*

The Pi in the 10 experimental plots cultivated in spring–summer ranged from 10 to 547 J2 per 250 cm^3 of soil, and the minimum, maximum and mean soil temperatures were 17.5, 28.2 and 23.5 °C, respectively. The Pi in the 15 experimental plots cultivated in autumn–winter ranged from 42 to 842 per 250 cm^3 of soil, and the minimum, maximum and mean soil temperatures were 6.8, 25.5 and 16.4 °C, respectively. The *S. torvum* rootstocks showed a lower ($P < 0.05$) galling index than the susceptible MC eggplant, with fewer ($P < 0.05$) eggs per plant, irrespective of the cropping season. The resistance level of the *S. torvum* rootstocks shifted from highly resistant or resistant in the spring–summer crop to moderately resistant in the autumn–winter crop. The maximum multiplication rate (a), maximum density (M) and equilibrium density (E) of *M. incognita* were higher on the susceptible MC than on the *S. torvum* rootstocks, irrespective of the cropping season (Table 3.5). The population growth rate of *M. incognita* in the MC differed from those in all the *S. torvum* rootstocks in the spring–summer crop (fig. 3.3a) (MC *vs* TB: intercept $P = 0.0009$, slope $P = 0.5829$; MC *vs* TE: intercept $P = 0.0095$, slope $P = 0.9909$; MC *vs* TS: intercept $P = 0.0009$, slope $P = 0.3637$; MC *vs* TT: intercept $P = 0.0018$, slope $P = 0.8036$), but only differed from some of them when cultivated in autumn–winter (fig. 3.3b) (MC *vs* TB: intercept $P = 0.0125$, slope $P = 0.0854$; MC *vs* TE: intercept $P = 0.0136$, slope $P = 0.1150$; MC *vs* TS: intercept $P = 0.0060$, slope $P = 0.0730$; MC *vs* TT: intercept $P = 0.4685$, slope $P = 0.9616$).

Table 3.1: Resistance and origin of *Solanum melongena* (eggplant) and the *S. torvum* rootstock cultivars used in experiments.

Plant host	Cultivar (code)	Resistance	Company
S. melongena	Cristal (MC)		Semillas fito
S. torvum	Brutus (TB)	HR: Ma, Mi, Mj; IR: Va, Vd	Semillas fito
	Espina (TB)	HR: Fom; Rs, Pl, Ma, Mi, Mj, Vd	Asasem
	Salutamu (TS)	HR: N, Pl, Vd	La perla de sud
	Torpedo (TT)	HR: Rs, V, Fol; IR: N	Ramiro Arnedo

[a]HR: high resistance; IR: intermediate resistance; Fom: *Fusarium oxysporum* f. sp. *melongena*; Fol: *Fusarium oxysporum* f.sp. *lycopersici*; Ma: *Meloidogyne arenaria*; Mi: *M. incognita*; Mj: *M. javanica*; N: Nematodes (*Meloidogyne*); Pl: *Pyrenochaeta lycopersici*; Rs: *Ralstonia solanacearum*; V: *Verticillium* spp.; Va: *Verticillium alboatrum* Vd: *Verticillium dahliae*.

Table 3.2: *Meloidogyne* isolates from Spain, geographic origin, virulence status against tomato cultivars carrying the *Mi1.2* of tomato or *N* of pepper.

Meloidogyne spp.	Isolate	Geographic origin	(a)Virulence	Referece
M. incognita	MIAd	Barcelona	Avirulent	-
	MIAm	Barcelona	Avirulent	-
	MIAL09	Almeria	Avirulent	Verdejo-Lucas et al. 2012
	MIAL30	Almeria	Avirulent	Verdejo-Lucas et al. 2012
	MIPa	Almeria	Avirulent	-
	MILGN	Almeria	*N*-virulent	-
M. javaniva	MJ05	Barcelona	Avirulent	Ornat et al. 2001
	MJAL01	Almeria	Avirulent	Verdejo-Lucas et al. 2012
	MJAL05	Almeria	Avirulent	Verdejo-Lucas et al. 2012
	MJPm	Murcia	Avirulent	-
	MJTU	Barcelona	Avirulent	Expósito et al. 2018
	MJVI	Barcelona	Avirulent	-
	MJ27	Barcelona	*Mi1.2*-virulent	Ornat et al. 2001
	MJLg	Almeria	*Mi1.2*-virulent	-

3.4 Discussion

The results of this study show that *S. torvum* is not a suitable host for most *Meloidogyne* isolates from Spain, according to the resistance levels registered. The mechanisms of resistance are not clear, but sesquiterpenoids and chitinases seem to play a key role, acting as nematotoxic and nematostatic compounds affecting the viability of the nematode at various stages (Bagnaresi et al. 2013).

Although all the nematode isolates tested reproduced less in the *S. torvum* cultivars than on the susceptible eggplant, the species of nematode influenced the level of resistance. Indeed, high resistance levels to *M. incognita* were found in both pot and plastic greenhouse experiments, similar to other results previously reported about *M. incognita* populations from other parts of the world (Daunay and Dalmasso 1985; Ali et al. 1992; Rahman et al. 2002; Dhivya et al. 2014; Uehara et al. 2017b). However, the host suitability of *S. torvum* to *M. javanica* varied greatly from resistant to susceptible, as in previous reports (Daunay and Dalmasso 1985; Tzortzakakis et al. 2006; Öçal et al. 2018). This variability in the plant–nematode interaction could be due to the genetic background of both plant germplasm and RKN isolate, as has been postulated for tomato rootstocks (Cortada et al. 2008). For example, in this study all the *S. torvum* cultivars responded with a moderately resistant to susceptible reaction to the *M. javanica* isolate MJ05 which reproduced 81% less than the isolate MJAl05, which achieved the higher reproduction in the susceptible eggplant cv. Cristal. In addition, the *S. torvum* rootstock Torpedo was more suitable to the most *M. javanica* isolates according to the reproduction index.

The effect of increasing *Pi* did not affect the *S. torvum* suitability. Lower values of maximum multiplication rate (a) and maximum population density (M) of both *M. javanica* and *M. incognita* on *S. torvum* than on the susceptible eggplant were estimated, confirming its host status. The cropping season influenced the population growth rate of all the *S. torvum* cultivars and of the eggplant. The lower resistance level of the *S. torvum* cultivars were observed when cropped during autumn–winter, mainly due to a more pronounced reduction of the population growth rate on the susceptible eggplant that seemed more affected than the *S. torvum* cultivars when cropped in this period. In fact, eggplant is a warm season crop affected by low temperatures.

Table 3.3: Number of eggs per plant and reproduction index (RI) of MJLg isolate of *Meloidogyne javanica* on the susceptible tomato, *Solanum lycopersicum*, cv. Durinta and the resistant cv. Monika, the susceptible pepper, *Capsicum annuum*, cv. Compás and the resistant cv. Solfeo 55 days after inoculation with a rate of 1 second-stage juvenile/cm^3.

Meloidogyne spp.	Isolate	Crop	Cultivar	Eggs per plant ($\times 10^2$)	RI (%)[a]
M. javanica	MJLg	Tomato	Durinta	477.4 ± 93.8	
			Monika	469.3 ± 51.5	98.3 ± 10.8
M. incognita	MILgN	Tomato	Durinta	387.9 ± 50.1*	
			Monika	10.2 ± 2.5	2.6 ± 0.6
		Pepper	Compas	63.9 ± 11.0	
			Solfeo	57.0 ± 15.5	89.3 ± 24.2

Data are mean ± standard error of 14 replicates. Values per each *Meloidogyne* species followed by * show significant differences ($P < 0.05$) between cultivars of the same crop according to the Mann–Whitney U non parametric test.
[a]Reproduction index = 100 × (number of eggs per plant in the resistant cultivar / mean number of eggs per plant on the susceptible cultivar).

Cultivar Brutus confers tolerance to *M. javanica* because the dry shoot weight of the grafted eggplant was not reduced when submitted to increasing *Pi* but that of the ungrafted did according to the Seinhorst's damage model. The estimated tolerance limit of eggplant to *M. javanica* was 10.9 J2 per 100 cm^3 of sandy soil. Di Vito et al. (1986) reported a tolerance limit of 0.054 eggs and J2 of *M. incognita* per cm^3 of sandy loam soil. Watanabe et al. (2014) reported eggplant yield losses at *Pi* higher than 2 J2 of a mixed population of *M. incognita* and *M. hapla* per 20 g of clay loam soil. It is known that the tolerance limit can be influenced by the plant cultivar, nematode species and densities, the source of inoculum, soil type and cropping system (Greco and Di Vito 2009). Therefore, an accurate estimation of the tolerance limit must be obtained for specific conditions in order to be used as a decision-making tool.

Interestingly, this study provides the first evidence of the resistance of *S. torvum* to a *N*-virulent *M. incognita* isolate. In addition, the resistance to *Mi1.2*-virulent isolates has been corroborated in agreement with that of the *S. torvum* cultivars Tonashimu, Torero and Torvum vigour to two *Mi1.2*-virulent *M. incognita* from Japan, and to that of cv. Hawk to a *Mi1.2*-virulent *M. incognita* from Turkey (Uehara et al. 2017a; Öçal et al. 2018).

This study points out that *S. torvum* is a valuable tool for managing *M. incognita* and *M. javanica* populations irrespective of their (a)virulence status to the resistant *N* and *Mi1.2* genes, and may help to avoid selection for virulence to any specific *R* gene in growing systems in which crop rotation is done. The inclusion of some more resistance sources in the rotation could prevent the selection to specific *R* genes because the high specificity of the virulence to a given *R* gene and the fitness cost that RKN population acquires, raises the possibility of counterselection by the proper use of different resistance sources (Djian-Caporalino et al. 2011). In fact, alternating only two resistance genes does not prevent the selection for virulence, although the level is reduced (Expósito et al. 2019). The number of resistance sources is expected to increase and to be available to growers in the future, as for example the accessions of *Cucumis metuliferus* and *C. amarus* recently characterized as resistant to *Mi1.2* (a)virulent isolates (Expósito et al. 2018; García-Mendívil et al. 2019).

Some other wild eggplant species, such as *S. aethiopicum*, *S. sisymbriifolium* and *S. warscewiczii*, are also resistant to the three tropical RKN species but less than *S. torvum* (Daunay and Dalmasso 1985). De-

Table 3.4: Number of eggs per plant and reproduction index (RI) of *Meloidogyne incognita* and *M. javanica* isolates on the eggplant cv. Cristal (MC) and the *Solanum torvum* rootstocks cv. Brutus (TB), Espina (TE), Salutamu (TS) and Torpedo (TT). Plants were inoculated with 1 J2/cm^3 of sand and maintained for 40 days (experiment 1), 49 days (experiment 3), or 55 days (experiment 2).

Experiment	*Meloidogyne* spp.	Isolate	Egg masses per plant($\times 10^2$)					RI (%)[a]			
			S. torvum				Eggplant	*S. torvum*			
			TB	TE	TS	TT	MC	TB	TE	TS	TT
Experiment 1	*M. incognita*	MIAd	0.7 ± 0.2 c D	3.6 ± 1.1 b DE	14.5 ± 5.2 bc BC	2.2 ± 0.5 b D	315.2 ± 74.9 a B	0.2 ± 0.1	1.1 ± 0.3	4.6 ± 1.6	0.7 ± 0.1
		MIAm	7.3 ± 2.6 b B-D	4.2 ± 0.9 b DE	4.8 ± 1.2 b CD	4.6 ± 1.8 b D	96.0 ± 21.8 a C-E	7.6 ± 2.7	4.4 ± 1	5.0 ± 1.2	4.8 ± 1.9
		MIAl09	5.2 ± 1.5 b CD	4.9 ± 2.9 b DE	2.6 ± 0.5 b CD	1.6 ± 0.4 b D	101.3 ± 30.3 a C-E	5.1 ± 1.5	4.9 ± 2.9	2.6 ± 0.5	1.6 ± 0.4
		MIAl30	10.4 ± 2.5 b A-C	27.2 ± 6.2 b BC	24.5 ± 6.6 b AB	4.9 ± 1.6 B D	269.1 ± 131.7 a BC	3.9 ± 0.9	10.1 ± 2.3	8.2 ± 2.4	1.8 ± 0.6
		MIPa	15.3 ± 3.6 b A	21.7 ± 10.6 b C	13.8 ± 4.7 b B-D	5.2 ± 1.7 B D	667.7 ± 125.4 a A	2.3 ± 0.5	3.2 ± 1.6	2.1 ± 0.7	0.8 ± 0.3
	M. javanica	MJ05	13.3 ± 2.2 bc AB	32.5 ± 6.2 b BC	21.4 ± 4.3 b AB	10.0 ± 2.5 c CD	61.5 ± 12.2 a DE	21.5 ± 3.5	52.9 ± 10.1	34.8 ± 7.1	16.2 ± 4.1
		MJAl01	10.4 ± 3.5 c A-C	17.1 ± 5.6 bc CD	12.8 ± 3.5 bc B-D	35.2 ± 10.0 b A	179.6 ± 38.8 a B-E	5.8 ± 2.0	8.5 ± 3.0	7.1 ± 1.9	19.6 ± 5.5
		MJAl05	15.1 ± 4.6 b A	40.7 ± 8.1 b A	19.5 ± 2.4 b AB	33.1 ± 7.5 b AB	318.8 ± 64.6 a B	4.7 ± 1.4	12.8 ± 2.5	6.1 ± 0.8	10.4 ± 2.3
		MJPm	0.1 ± 0.1 c D	0.3 ± 0.3 c E	0.8 ± 0.2 b D	2.2 ± 1.7 bc D	7.2 ± 1.8 a E	1.8 ± 1.8	4.0 ± 4.0	11.0 ± 3.4	30.4 ± 23.1
		MJTu	3.3 ± 1.6 bc CD	1.6 ± 0.7 c DE	1.0 ± 0.4 c D	9.0 ± 2.9 b D	36.8 ± 9.6 a E	9.0 ± 4.3	4.4 ± 1.9	2.6 ± 1.0	24.4 ± 7.8
		MJVi	9.3 ± 2.3 b A-C	41.9 ± 7.8 b A	29.3 ± 11.2 b A	22.2 ± 5.4 b BC	231.3 ± 82.9 a B-D	4.0 ± 1.0	18.1 ± 3.4	12.7 ± 4.8	9.6 ± 2.3
Experiment 2	*M. javanica*	MJLg	2.2 ± 1.2 b	0.4 ± 0.1 b	2.6 ± 1.3 b	12.4 ± 4.8 b	65.5 ± 9.8 a	3.3 ± 1.9	0.5 ± 0.1	3.9 ± 2.0	19.0 ± 7.3
Experiment 3	*M. incognita*	MILgN	6.9 ± 1.9 b				645.3 ± 102.6 a	1.1 ± 0.3			
	M. javanica	MJ27	56.2 ± 9.6 b				729.0 ± 131.7 a	7.7 ± 1.3			

Data are mean ± standard error of 14 replicates. Data within the same row followed by the same lowercase letter did not show significant differences ($P < 0.05$) between germplasm according to the Dunn test. Different uppercase letters in the same column indicate significant differences ($P < 0.05$) between nematode isolates according to the Dunn test.

[a]Reproduction index = 100 × (number of eggs per plant in the *S. torvum* cultivar / number of eggs per plant on the eggplant cv. Cristal).

Table 3.5: Galling index (GI), number of eggs/plant, reproduction index (RI), maximum multiplication rate (a), maximum population density (M, J2+eggs/250 cm^3 of soil) and equilibrium density (E, J2+eggs/250 cm^3 soil) of *M. incognita* on the eggplant cv. Cristal (MC) and the *S. torvum* rootstocks cv. Brutus (TB), Espina (TE), Salutamu (TS) and Torpedo (TT) cultivated in plastic greenhouse from May 10 to June 27 2016 (spring–summer) in plots with increasing nematode densities at transplantation (Pi: 10–547 J2/250 cm^3 soil), and from October 4 2016 to January 31 2017 (autumn–winter) (Pi: 42–842 J2/250 cm^3 soil).

		Sping-summer						Autumn-winter					
Plant host	Cultivar	Gi[a]	Eggs per plant($\times 10^2$)	RI[b]	a	M	E	GI[a]	Eggs per plant($\times 10^2$)	RI*b*	a	M	E
Eggplant	MC	4.7 ± 0.3 a	808 ± 205 a		27450	2462400	451346	2.9 ± 0.3 a	16.3 ± 5.0 a		1105	95000	2431
S. torvum	TB	1.7 ± 0.3 b	6.0 ± 2.1 b	1.0 ± 0.3	50	21560	6438	1.6 ± 0.2 b	1.6 ± 0.3 b	22.6 ± 10.9	67	6407	3389
	TE	2.0 ± 0.3 b	8.8 ± 1.7 b	1.5 ± 0.3	253	18352	8411	1.6 ± 0.2 b	1.3 ± 0.3 b	11.8 ± 3.3	72	7380	1308
	TS	1.5 ± 0.2 b	7.1 ± 1.6 b	1.8 ± 0.7	106	16340	23622	1.6 ± 0.1 b	1.1 ± 0.2 b	18.3 ± 9.3	29	2850	1867
	TT	1.7 ± 0.3 b	5.0 ± 1.3 b	0.9 ± 0.2	87	13680	4559	1.7 ± 0.1 b	2.8 ± 0.8 b	20.8 ± 5.5	187	7800	804

Data are mean ± standard error of 10 and 15 replicates in the spring–summer and autumn–winter experiments, respectively. Data in the same column followed by different letters indicate significant differences ($P < 0.05$) between plant cultivars according to the Dunn test.

[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck 1971).

[b]Reproduction index = 100 × (number of eggs per plant in a given *S. torvum* rootstock / that on the susceptible eggplant).

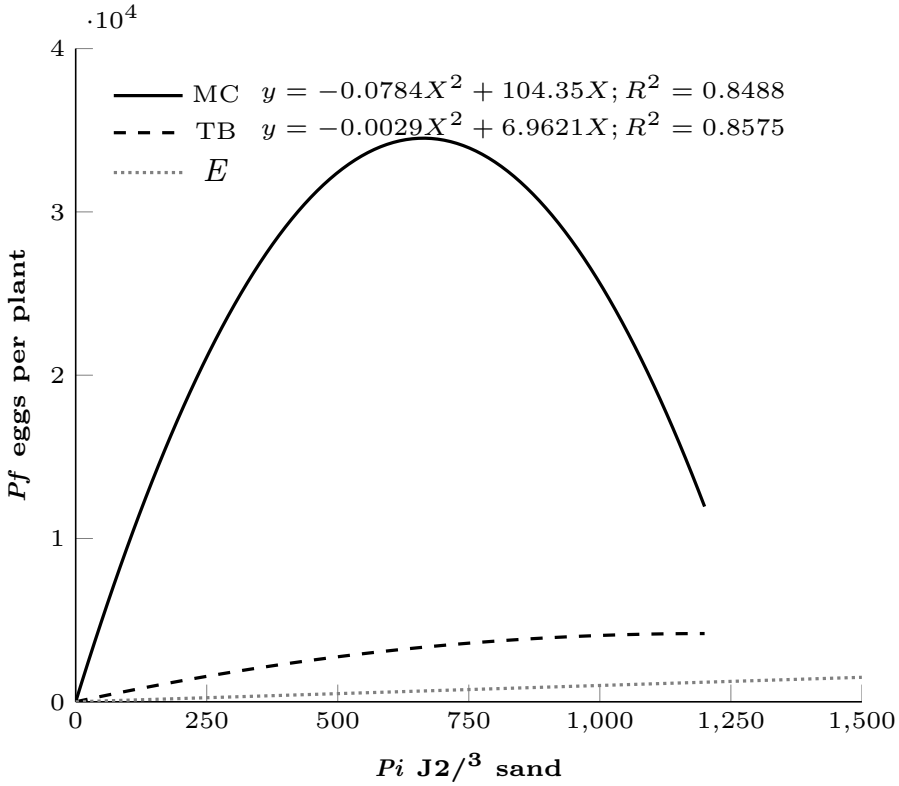


Figure 3.1: Relationship between initial (*Pi*) and final (*Pf*) *Meloidogyne javanica* densities in ungrafted eggplant cv. Cristal (MC) and grafted onto the *S. torvum* rootstock cv. Brutus (TB) cultivated in 500 cm^3 pots inoculated with increasing inoculum levels of second-stage juveniles (J2) and maintained in a greenhouse for 55 days, from June to August 2017. Each point is the mean of 4 replicates.

spite the value of *S. torvum* as a rootstock, it is not yet widely used because of its poor and irregular seed germination, making it necessary to sow it 20–30 days earlier than the scion (Oda and Lee 2003). However, seed treatments that result in a high and rapid germination (Ranil et al. 2015) could favour its use in the future.

Acknowledgments

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R and AGL2017-89785-R, and CONACYT for providing PhD funding to H. A. García-Mendvíl. Thank are also given to Manuel López- Gómez for providing the *M. javanica* MJLg and *M. incognita* MILgN populations, Manuel López-Gómez and Adriana Iglesias Culebras from Rijk Zwaan Iberica for providing the pepper seeds, Laura Mateo from Semillas fitó for providing Brutus seeds, and to Sergi García, Alejandro Expósito, Anna Sanz and Miquel Masip for technical assistance. The authors have no conflict of interest to declare.

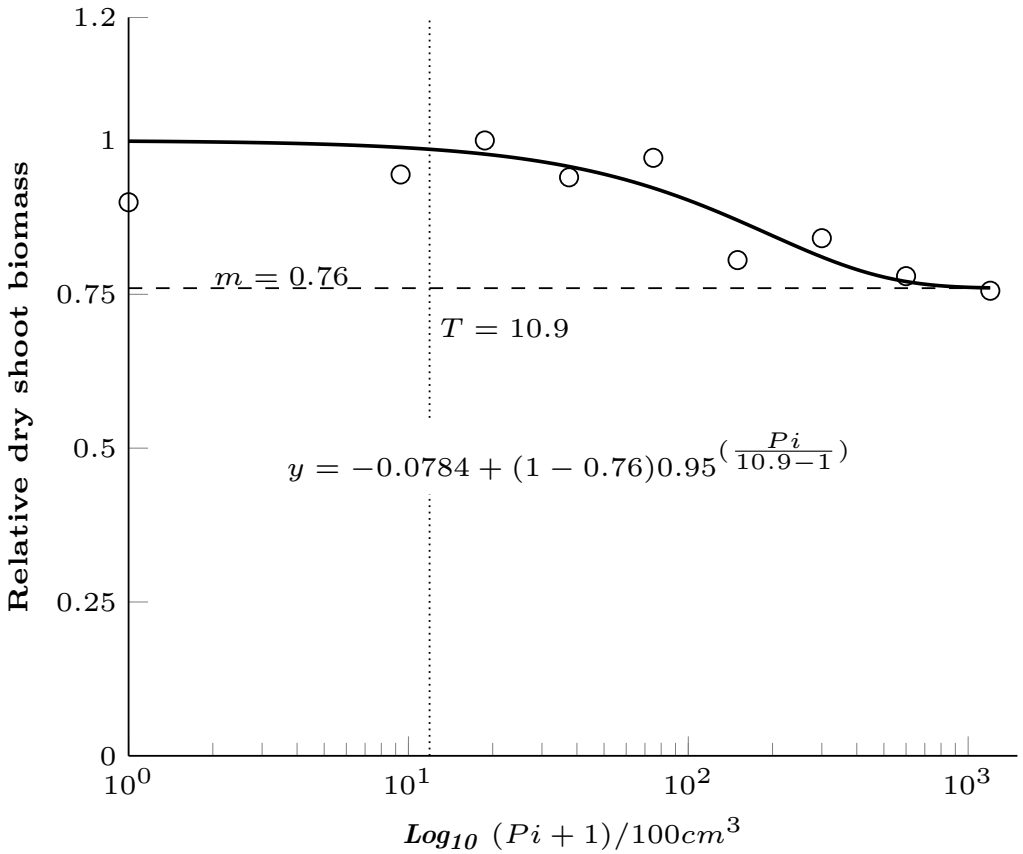


Figure 3.2: Relationship between *Meloidogyne javanica* initial population densities (Pi) and relative dry top weight of eggplant cv. Cristal cultivated in 500 cm^3 pots inoculated with increasing inoculum levels of second-stage juveniles (J2) and maintained in a greenhouse for 55 days, from June to August 2017. Each point is the mean of 4 replicates.

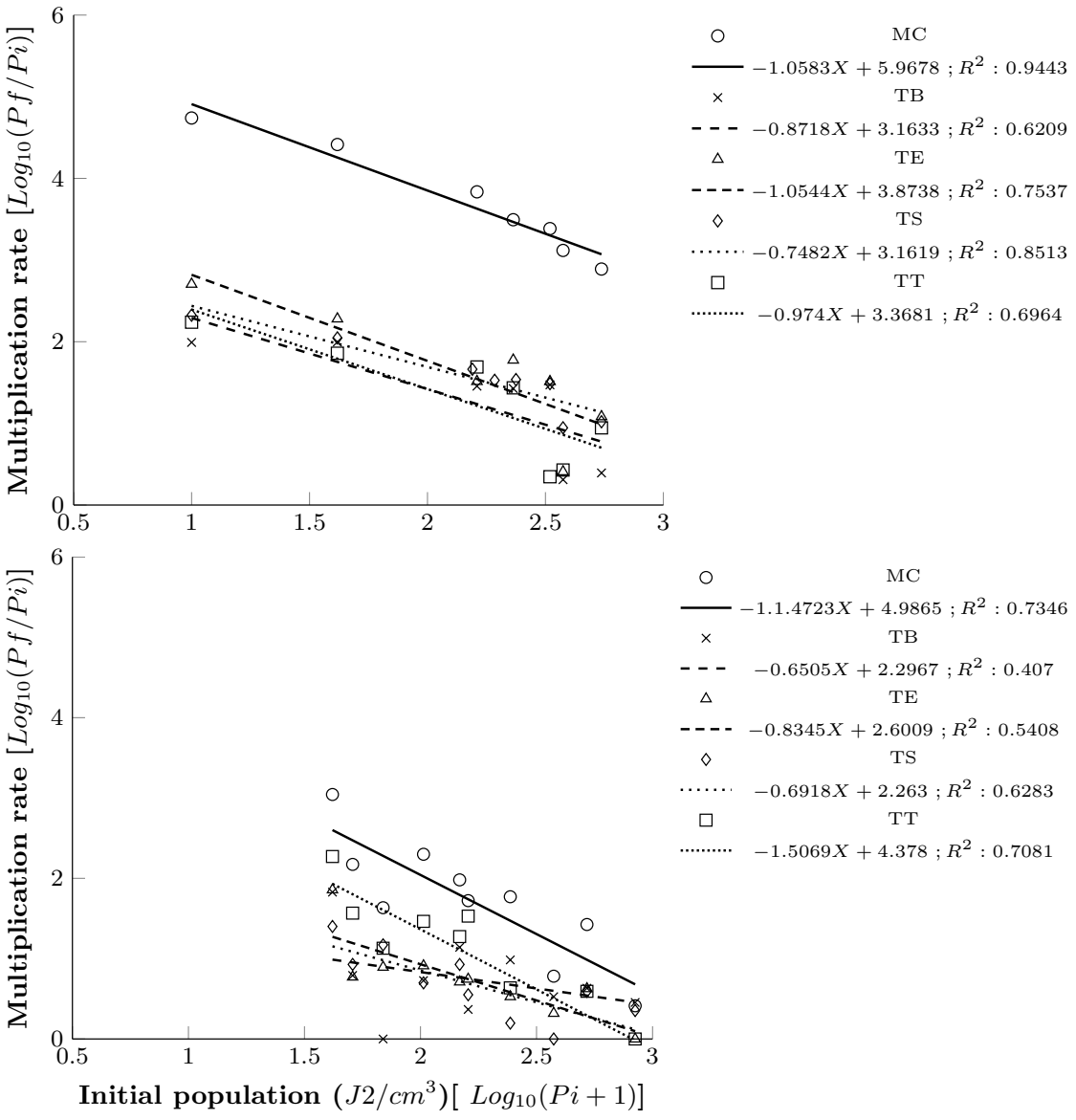


Figure 3.3: Relationship between initial population density (*Pi*) and multiplication rate (*Pf/Pi*) of *Meloidogyne incognita* on the eggplant cv. Cristal (MC) and the *S. torvum* rootstock cultivars Brutus (TB), Espina (TE), Salutamu (TS) and Torpedo (TT), cultivated in a plastic greenhouse from May 10 to June 27 2016 (spring–summer) in 10 plots with increasing nematode densities at transplantation (*Pi*: 10–547 J2/250 cm^3 of soil), and from October 4 2016 to January 31 2017 in 15 plots (autumn–winter) (*Pi*: 42–842 J2/250 cm^3 of soil).

References

Ali, M., N. Matsuzoe, H. Okubo, and K. Fujieda. 1992. Resistance of Non-tuberous *Solanum* to Root-knot Nematode. *Horticultural science* 60 (4): 921–926. ISSN: 1880-358X. doi:10.2503/jjshs.60.921. http://joi.jlc.jst.go.jp/JST.Journalarchive/jjshs1925/60.921?from=CrossRef.

Araujo, M. T., M. J. Bassett, J. J. Augustine, and D. W. Dickson. 1982. Effects of the temperature and duration of the initial incubation period on resistance to *Meloidogyne incognita* in tomato. *Journal of nematology* 14 (3): 411.

Bagnaresi, P., T. Sala, T. Irdani, C. Scotto, A. Lamontanara, M. Beretta, et al. 2013. *Solanum torvum* responses to the root-knot nematode *Meloidogyne incognita*. *BMC Genomics*. ISSN: 14712164. doi:10.1186/1471-2164-14-540.

Cortada, L., F. J. Sorribas, C. Ornat, I. Kaloshian, and S. Verdejo-Lucas. 2008. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathology* 57, no. 6 (): 1125–1135. ISSN: 00320862. doi:10.1111/j.1365-3059.2008.01906.x. http://doi.wiley.com/10.1111/j.1365-3059.2008.01906.x.

Daunay, M.-C. 2008. Eggplant. In *Vegetables II*, ed. by J. Prohens and F. Nuez, 163–220. New York: Springer. doi:10.1007/978-0-387-74110-9_5. http://link.springer.com/10.1007/978-0-387-74110-9%7B%5C_%7D5.

Daunay, M.-C., and A. Dalmasso. 1985. Multiplication de *Meloidogyne javanica*, *M. incognita* et *M. arenaria* sur divers Solarium (’). *Revue Nématol* 8 (1): 31–34.

Dhivya, R., A. Sadasakthi, and M. Sivakumar. 2014. Response of wild *Solanum* rootstocks to root-knot nematode (*Meloidogyne incognita* Kofoid and White). *International Journal of Plant Sciences* 9 (1): 117–122.

Di Vito, M., N. Greco, and A. Carella. 1986. Effect of *Meloidogyne incognita* and importance of the inoculum on the yield of eggplant. *Journal of Nematology* 18 (4): 487.

Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, et al. 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *European Journal of Plant Pathology* 131, no. 3 (): 431–440. ISSN: 0929-1873. doi:10.1007/s10658-011-9820-4. http://link.springer.com/10.1007/s10658-011-9820-4.

Expósito, A., S. García, A. Giné, N. Escudero, and F. J. Sorribas. 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato–melon rotation sequence. *Pest Management Science* 75, no. 7 (): 1902–1910. ISSN: 1526-498X. doi:10.1002/ps.5297. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.5297.

Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, M. B. Picó, et al. 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67, no. 5 (): 1161–1167. ISSN: 00320862. doi:10.1111/ppa.12815. http://doi.wiley.com/10.1111/ppa.12815.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Statistics Division. Visited on 09/04/2019. http://www.fao.org/faostat.

García-Mendívil, H. A., M. Munera, A. Giné, N. Escudero, M. B. Picó, C. Gisbert, et al. 2019. Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. *Crop Protection* 119 (): 208–213. ISSN: 02612194. doi:10.1016/j.cropro.2019.02.005. https://linkinghub.elsevier.com/retrieve/pii/S0261219419300432.

Giné, A., and F. J. Sorribas. 2017. Effect of plant resistance and BioAct WG (*Purpureocillium lilacinum* strain 251) on *Meloidogyne incognita* in a tomato-cucumber rotation in a greenhouse. *Pest Management Science* 73, no. 5 (): 880–887. ISSN: 1526498X. doi:10.1002/ps.4357. http://doi.wiley.com/10.1002/ps.4357.

Greco, N., and M. Di Vito. 2009. 11 Population Dynamics and Damage Levels, ed. by R. Perry, M. Moens, and J. Starr, 246. CABI.

Hadisoeganda, W. W. 1982. Resistance of Tomato, Bean, Southern Pea, and Garden Pea Cultivars to Root-Knot Nematodes Based on Host Suitability. *Plant Disease* 66 (1): 145. ISSN: 0191-2917. doi:10.1094/PD-66-145. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1982Abstracts/PD%7B%5C_%7D66%7B%5C_%7D145.htm.

Hussey, R. S., and K. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025–1028.

Jones, J. T., A. Haegeman, E. G. J. Danchin, H. S. Gaur, J. Helder, M. G. K. Jones, et al. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*. ISSN: 14646722. doi:10.1111/mpp.12057.

Kumar, S., N. Bharti, and S. N. Saravaiya. 2018. Vegetable Grafting: A Surgical Approach to combat biotic and abiotic stresses: A review. *Agricultural Reviews*. ISSN: 0253-1496. doi:10.18805/ag.r-1711.

Öçal, S., T. Özalp, and Z. Devran. 2018. Reaction of wild eggplant *Solanum torvum* to different species of root-knot nematodes from Turkey. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/s41348-018-0167-3.

Oda, M., and J.-M. Lee. 2003. Grafting of Herbaceous Vegetable and Ornamental Crops. In *Horticultural Reviews*, 61–124. Oxford, UK: John Wiley & Sons, Inc. doi:10.1002/9780470650851.ch2. http://doi.wiley.com/10.1002/9780470650851.ch2.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 2001. A Population of *Meloidogyne javanica* in Spain Virulent to the *Mi* Resistance Gene in Tomato. *Plant Disease* 85, no. 3 (): 271–276. ISSN: 0191-2917. doi:10.1094/PDIS.2001.85.3.271. http://apsjournals.apsnet.org/doi/10.1094/PDIS.2001.85.3.271.

Rahman, M. A., M. A. Rashid, M. A. Salam, M. A. T. Masud, A. S. M. H. Masum, and M. M. Hossain. 2002. Performance of Some Grafted Eggplant Genotypes on Wild *Solanum* Root Stocks Against Root-Knot Nematode. *Journal of Biological Sciences* 2, no. 7 (): 446–448. ISSN: 17273048. doi:10.3923/jbs.2002.446.448. http://www.scialert.net/abstract/?doi=jbs.2002.446.448.

Ranil, R. H., H. M. Niran, M. Plazas, R. M. Fonseka, H. H. Fonseka, S. Vilanova, et al. 2015. Improving seed germination of the eggplant rootstock *Solanum torvum* by testing multiple factors using an orthogonal array design. *Scientia Horticulturae*. ISSN: 03044238. doi:10.1016/j.scienta.2015.07.030.

Robertson, L., J. A. López-Pérez, A. Bello, M. A. Díez-Rojo, M. Escuer, A. Piedra-Buena, et al. 2006. Characterization of *Meloidogyne incognita*, *M. arenaria* and *M. hapla* populations from Spain and Uruguay parasitizing pepper (*Capsicum annuum* L.) *Crop Protection*. ISSN: 02612194. doi:10.1016/j.cropro.2005.07.008.

Schomaker, C. H., and T. H. Been. 2013. Plant growth and population dynamics. In *Plant nematology*, ed. by R. Perry and M. Moens, 275–301. Ed. 2. Wallingford: CABI. doi:10.1079/9781845930561.0275. http://www.cabi.org/cabebooks/ebook/20063106966.

Seinhorst, J. W. 1970. Dynamics of Populations of Plant Parasitic Nematodes. *Annual Review of Phytopathology* 8, no. 1 (): 131–156. ISSN: 0066-4286. doi:10.1146/annurev.py.08.090170.001023. http://www.annualreviews.org/doi/10.1146/annurev.py.08.090170.001023.

— . 1998. The common relation between population density and plant weight in pot and microplot experiments with various nematode plant combinations. *Fundamental and Applied Nematology* 21:459–468. ISSN: 1164-5571. http://www.documentation.ird.fr/hor/fdi:010017629.

Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano, and J. Valero. 2005. Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology* 111, no. 1 (): 29–38. ISSN: 0929-1873. doi:10.1007/s10658-004-1982-x. http://link.springer.com/10.1007/s10658-004-1982-x.

Talavera, M., S. Verdejo-Lucas, C. Ornat, J. Torres, M. D. Vela, F. J. Macias, et al. 2009. Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses. *Crop Protection* 28, no. 8 (): 662–667. ISSN: 02612194. doi:10.1016/j.cropro.2009.03.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219409000763.

Tzortzakakis, E. A., F. A. Bletsos, and A. D. Avgelis. 2006. Evaluation of *Solanum* rootstock accessions for control of root-knot nematodes and tobamoviruses. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/BF03356178.

Uehara, T., M. Sakurai, K. Oonaka, Y. Tateishi, T. Mizukubo, and K. Nakaho. 2017a. Reproduction of *Meloidogyne incognita* on eggplant rootstock cultivars and effect of eggplant rootstock cultivation on nematode population density. *Nematological Research (Japanese Journal of Nematology)*. ISSN: 0919-6765. doi:10.3725/jjn.46.87.

Uehara, T., Y. Tateishi, Y. Kadota, and H. Iwahori. 2017b. Differences in parasitism of *Meloidogyne incognita* and two genotypes of *M. arenaria* on *Solanum torvum* in Japan. *Journal of Phytopathology*. ISSN: 14390434. doi:10.1111/jph.12594.

Verdejo-Lucas, S., L. Cortada, F. J. Sorribas, and C. Ornat. 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of mi resistance gene tomato rootstocks under field conditions. *Plant Pathology*. ISSN: 00320862. doi:10.1111/j.1365-3059.2009.02089.x.

Verdejo-Lucas, S., M. Talavera, and M. F. Andrés. 2012. Virulence response to the *Mi.1* gene of *Meloidogyne* populations from tomato in greenhouses. *Crop Protection*. ISSN: 02612194. doi:10.1016/j.cropro.2012.03.025.

Watanabe, T., H. Masumura, Y. Kioka, K. Noguchi, Y. Y. Min, R. Murakami, et al. 2014. Development of a direct quantitative detection method for *Meloidogyne incognita* and *M. hapla* in andosol and analysis of relationship between the

initial population of *Meloidogyne* spp. and yield of eggplant in an andosol. *Nematological Research*. ISSN: 0919-6765. doi:10.3725/jjn.43.21.

Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, no. 1 (): 25–38. ISSN: 0003-4746. doi:10.1111/j.1744-7348.1965.tb07864.x. http://doi.wiley.com/10.1111/j.1744-7348.1965.tb07864.x.

Zeck, W. M. 1971. Rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschutz nachrichten*, no. 24: 141–144. ISSN: 0079-1342.

Zijlstra, C., D. T. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2 (8): 847–853. ISSN: 1388-5545. doi:10.1163/156854100750112798. https://brill.com/view/journals/nemy/2/8/article-p847%7B%5C_%7D3.xml.

Chapter 4

Fitness cost but no selection for virulence in *Meloidogyne incognita* after two consecutive crops of eggplant grafted onto *Solanum torvum*

Fitness cost but no selection for virulence in *Meloidogyne incognita* after two consecutive crops of eggplant grafted onto *Solanum torvum*

Helio A.García-Mendívil and Francisco J. Sorribas

2019. *Plant Pathology*, ppa.13092. https://doi.org/10.1111/ppa.13092

Abstract

The eggplant *Solanum melongena* cv. Cristal, either ungrafted or grafted onto the *S. torvum* rootstock cv. Brutus was cultivated for two consecutive years in the same plots in a plastic greenhouse to assess the level of resistance to *M. incognita* and crop yield. At the end of the second crop, the putative selection for virulence of the nematode subpopulations coming from infected ungrafted and grafted eggplant was assessed in the eggplant and in *S. torvum* in a pot experiment. Nematode population densities at transplantation in 2017 ranged from 2 to 378 J2 per 100 cm^3 of soil and did not differ between ungrafted and grafted eggplant. At the end of each crop, higher galling index and number of nematodes in soil and in roots were registered in ungrafted than grafted eggplant. The grafted eggplant performed as resistant in 2017 and as highly resistant in 2018. Eggplant yield did not differ irrespective of grafting in 2017 after being cultivated for 135 days, but it differed after 251 days of cultivation in 2018. In the pot experiment, *S. torvum* performed as resistant to both *M. incognita* subpopulations. However, the *M. incognita* subpopulation obtained from roots of *S. torvum* produced 49.4% less egg masses and 56% less eggs per plant in the eggplant than the nematode subpopulation obtained from roots of the eggplant cv. Cristal. The results of this study revealed that the infective and reproductive fitness of the nematode decrease without having been selected for virulence.

4.1 Introduction

Eggplant,*Solanum melongena*, is one of the most cultivated solanaceous crops with an estimated worldwide production of c. 52 million tonnes in 1.8 million ha (FAOSTAT 2017). Root-knot nematodes (RKN), *Meloidogyne* spp., are one of the most damaging soilborne pathogens in solanaceous crops, especially under protected cultivation (Hallmann and Meressa 2018). Maximum eggplant yield losses of 95% have been reported (Greco and Di Vito 2009). The use of resistant plants is an effective and economically profitable management strategy to control RKN (Sorribas et al. 2005) that is environmentally friendlier than the common soil nematicides (Nyczepir and Thomas 2009). In Nematology, plant resistance is defined as the ability of a plant to supress infection development and/or reproduction of plant-parasitic nematodes (Roberts 2009). Grafting onto resistant rootstocks has become a common method to control soilborne pathogens when no commercial resistant cultivars are available (Oda 2002; Thies et al. 2015). That is the case of eggplant, which has been usually grafted onto resistant tomato or interspecific hybrids such as *S. lycopersicum* × *S. habrochaites* (Daunay 2008). However, the expression of resistance can be limited by several factors like constant soil temperatures above 30 °C (Araujo et al. 1982), and the genetic background of the rootstock along with that of the nematode species (Cortada et al. 2008). Moreover, the repeated cultivation of plant species carrying the same resistance gene can select virulent nematode populations capable to overcome the plant defense mechanisms (Verdejo-Lucas et al. 2009; Giné and

Sorribas 2017; Expósito et al. 2019). Thus, other resistance sources have been assessed, including *S. melongena* lines, interspecific hybrid of *S. integrifolium* × *S. melongena*, and the wild related species *S. integrifolium*, *S. sisymbriifolium* and *S. torvum* (Daunay 2008). Nonetheless, *S. torvum* is currently the only wild species commercially available to be used as rootstock for eggplant worldwide (Uehara et al. 2017; Öçal et al. 2018). Several *S. torvum* accessions and cultivars have been previously described as resistant to *M. incognita* (Ali et al. 1992; Rahman et al. 2002; Dhivya et al. 2014), *M. luci* (Öçal et al. 2018), and to some populations of *M. arenaria* and *M. javanica* (Tzortzakakis et al. 2006; Uehara et al. 2017; Öçal et al. 2018); but susceptible to M. hapla (Öçal et al. 2018). Some *S. torvum* rootstocks have been recently described as a valuable tool for managing *M. incognita* and *M. javanica* populations from Spain, irrespective of their (a)virulence status to the resistance *N* and *Mi1.2* genes in pepper and tomato, respectively (García-Mendívil et al. 2019b). Nevertheless, no information about the effect of the continuous cultivation of *S. torvum* on selecting for virulence and on the nematode fitness has been previously reported. Therefore, experiments were conducted to estimate the effect of two-year cultivation in the same plot under plastic greenhouse conditions on *M. incognita* population densities, disease severity, eggplant yield, selection for virulence and nematode fitness.

4.2 Materials and methods

4.2.1 Effect of *S. torvum* on *M. incognita* reproduction, disease severity and eggplant yield cultivated in plastic greenhouse

The experiment was conducted in a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain). The soil texture was sandy loam with 83.8% sand, 6.7% loam and 9.5% clay; pH 8.7; 1.8% of organic matter (w/w) and 0.5 dS/m electrical conductivity. The soil was infested in 2014 with the avirulent *Mi1.2* gene isolate Agropolis from *M. incognita* coming from a single egg mass and multiplied in the susceptible tomato cv. Durinta (Expósito et al. 2019). The plots used in the experiment were previously cultivated with the rotation lettuce-French bean-eggplant. The experiment consisted of two treatments: the eggplant cv. Cristal (Semillas Fitó) grafted onto the *S. torvum* cv. Brutus (Semillas Fitó), and the ungrafted eggplant cv. Cristal as standard for comparison. Each treatment was replicated 10 times in plots with a narrow variation on nematode densities between treatments at transplantation. Crops were carried out from June 16 to October 29 2017 (135 d) and from March 20 to November 26 2018 (251 d) and plots maintained in black fallow between cropping seasons. Individual plots consisted of a row of 2.5 m with 4 plants spaced 0.6 m apart plots within a row were spaced 1 m. Plants of each treatment were cultivated in the same plot each year to determine the effect on *M. incognita* population densities, the disease severity, the crop yield and the durability of the resistance. Soil of each plot was prepared individually to avoid cross contamination. Plants were irrigated as needed trough a drip irrigation system and fertilized with a solution of NPK (15-5-30) at 31 kg/ha, iron chelate and micronutrients at 0.9 kg/ha. Weeds were removed manually before and during the cropping season. Fruit yield were determined weekly between 8 and 17 weeks after transplantation in 2017, and between 11 and 31 weeks in 2018. Soil temperatures and water content were recorded daily at 1 h intervals with digital probes 5TM (Decagon devices, Inc.) placed at 15 cm depth.

The initial nematode populaion densities(Pi) in soil were quantified at transplantation and the final population densities (Pf) at the end of the crops. Soil samples were taken from each experimental plot and consisted of eight cores taken from the first 30 cm of soil with an auger of diameter 2.5 cm that were mixed and passed through a 4 mm pore sieve to remove stones. The second-stage juveniles (J2) were extracted

from 500 cm^3 of soil and incubated at 27 ± 2 °C for one week, using Baermann trays (Whitehead and Hemming 1965). J2 were collected using a 25 μm aperture screen, counted, and expressed as J2/100 cm^3 of soil. At the end of the crop, roots were carefully uprooted, washed, and the galling index (GI) evaluated on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots dead (Zeck 1971). After that, the number of eggs/plant was assessed extracting them from roots by blender maceration in a 5% commercial bleach solution (Hussey and Barker 1973) and counted. Reproduction index (RI) was calculated as the percentage of eggs produced in the rootstock with regard to that in the eggplant cultivar. The response of the rootstock was categorized according to the RI as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Hadisoeganda 1982).

4.2.2 Selection for virulence and nematode fitness

At the end of the plastic greenhouse experiment in 2018, two nematode subpopulations were considered according to the plant species in which they were produced, that is, eggplant or *S. torvum*. The eggs extracted from roots of the ungrafted eggplant were inoculated in Baermann trays at 27 ± 2 °C to allow J2 emergence to determine the putative selection for virulence in a pot experiment. J2 emerged in the first 24 h were discarded. Nematodes were collected daily for 10 days using a 25 μm sieve, and stored at 9 °C until inoculation. Seeds of the *S. torvum* cv. Brutus were pretreated with a KNO_3 solution to improve germination (Ranil et al. 2015), transferred to vermiculite filled trays and incubated in a growth chamber at 25 ± 2 °C and 16:8h (light:dark) photoperiod for four weeks. Afterwards, the seedlings were transplanted to 200 cm^3 pots containing sterile sand and maintained in a growth chamber at 25 ± 2 °C with a 16:8 h (light:dark) photoperiod for a week, and inoculated with 1 J2/cm^3 sand. Each treatment was replicated 10 times. Plants were maintained in the growth chamber for 55 days. Plants were watered as needed throughout the experiment and fertilized with a slow-release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with PT100 probes (Campbell Scientific Ltd).

At the end of the experiments, the roots were carefully washed, the GI evaluated, the number of egg masses and egg/plant determined, the number of eggs/egg mass and RI calculated, and the level of resistance categorized following the procedures previously stated.

4.2.3 Statistical analysis

Statistical analyses were performed using R Statistical software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The data were not normally distributed according to the normal Shapiro–Wilk W test. Then, the nonparametric analyses Mann–Whitney U test was used for paired comparisons between plant species per cropping season, between plant species per each *M. incognita* subpopulation and between *M. incognita* subpopulations per each plant species.

4.3 Results

4.3.1 Effect of *S. torvum* on *M. incognita* reproduction, disease severity and eggplant yield cultivated in plastic greenhouse

The minimum and maximum soil temperatures during the cropping season in 2017 ranged from 21.1 °C to 29.9 °C, and from 14.6 °C to 31.3 °C in 2018. The nematode population densities in soil at transplantation in 2017 ranged from 2 to 378 J2/100 cm^3 of soil and did not differ ($P < 0.05$) between treatments. At the end of the crop, higher ($P < 0.05$) GI, and number of J2 in soil and eggs per plant were registered in the ungrafted than the grafted eggplant, but fruit yield did not differ (Table 4.1). In 2018,

Table 4.1: *Meloidogyne incognita* population densities in soil at transplantation (*Pi*) and at the end of the crop (*Pf*), galling index (GI), number of eggs/plant, reproduction index (RI) and eggplant, *S. melongena*, cv. Cristal yield ungrafted (MC) or grafted onto the *S. torvum* rootstock cv. Brutus (TB) cultivated from June to October 2017 (135 days) and March to November 218 (251 days) in the same plots in plastic greenhouse.

	J2 per 100 cm^3 soil					
Plant	*Pi*	*Pf*	GI[a]	Eggs per plant ($\times 10^2$)	RI (%)[b]	Yield (kg/plant)
First crop						
Ungrafted	51 ± 36	29054 ± 8626	4.6 ± 0.3	12323 ± 2408		0.8 ± 0.1
Grafted	28 ± 12	2061 ± 818*	1.0 ± 0.1*	228 ± 76*	2.0 ± 1.00	0.8 ± 0.1
Second crop						
Ungrafted	686 ± 302	821 ± 179	7.5 ± 0.2	36422 ± 5895		1.9 ± 0.2
Grafted	127 ± 54*	124 ± 44*	0.9 ± 0.1*	883 ± 138*	0.1 ± 0.01	4.0 ± 0.4*

Data on nematode population densities in soil are the mean ± standard error of 10 replicates. Data on GI, eggs/plant, RI and yield are the mean ± standard error of 40 replicates. Data followed by * in the same column and year indicate significant differences ($P < 0.05$) between plant species according to the non-parametric Mann–Whitney *U* test.
[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck, 1971).
[b]Reproduction index) = 100 × (number of eggs per plant in the rootstock / number of eggs per plant on the eggplant cv. Cristal).

the nematode population densities in soil at transplantation were between 94 and 98% less than those registered at the end of the crop in 2017 but differed ($P < 0.05$) between treatments. At the end of the crop, a higher ($P < 0.05$) GI and number of J2 in soil and eggs in roots were also registered in ungrafted than grafted eggplant. Grafted eggplant yielded 2.1 more kg of fruits per plant ($P < 0.05$) than ungrafted. The *S. torvum* rootstock performed as resistant (1% ≤ RI < 10%) to *M. incognita* in 2017, and highly resistant (RI < 1%) in 2018 (Table 4.1).

4.3.2 Virulence selection

The *S. torvum* cv. Brutus was resistant (1% ≤ RI < 10%) to both *M. incognita* subpopulations obtained from roots of the ungrafted eggplant cv. Cristal or grafted onto the *S. torvum* cv. Brutus after being cultivated for two consecutive years in the same plots in a plastic greenhouse. Both *M. incognita* subpopulations caused between 53 and 69% lower ($P < 0.05$) GI and produced 97% less egg masses and eggs per plant, and between 21 and 31% less eggs per egg mass in *S. torvum* than in eggplant (Table 4.2). The *M. incognita* subpopulation from roots of *S. torvum* cv. Brutus produced 49.4% less ($P < 0.05$) egg masses and 56% less eggs per plant in the eggplant cv. Cristal than the nematode subpopulation from roots of eggplant cv. Cristal, but these parameters did not differ between subpopulations when inoculated in *S. torvum* (Table 4.2).

Table 4.2: Galling index (GI), number of egg masses per plant, number of eggs per plant, number of eggs per egg mass and reproduction index (RI) of *Meloidogyne incognita* subpopulations obtained from roots of ungrafted eggplant, *S. melongena* cv. Cristal, and grafted onto the *S. torvum* rootstocks cv. Brutus in Cristal and Brutus 55 days after cultivation in 200 cm^3 pots inoculated with 1 J2/cm^3.

	GI[a]		Egg masses per plant		Eggs per plant		RI (%)[b]		Eggs per egg mass	
Plant	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
Cristal	3.8 ± 0.2	2.9 ± 0.2+	79 ± 9	40 ± 3+	39746 ± 4392	17526 ± 2084+			533 ± 47	440 ± 27
Brutus	1.8 ± 0.3*	0.9 ± 0.2*+	2 ± 1*	1 ± 0*	884 ± 230*	532 ± 138*	2.2 ± 0.6	3 ± 0.8	364 ± 55*	347 ± 103*

The nematode inoculum was obtained after cultivation the ungrafted and grafted eggplant cv. Cristal onto the rootstock cv. Brutus during two consecutive cropping seasons in the same plots in plastic greenhouse.

Data are mean ± standard error of 10 replicates. Values followed by * in the same column show significant differences ($P < 0.05$) between plant species according to the non-parametric Mann–Whitney U test. Values of each parameter followed by + show significant differences ($P < 0.05$) between nematode subpopulations per each plant specie according to the non-parametric Mann–Whitney U test.

[a]Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck, 1971).

[b]Reproduction index = 100 × (number of eggs per plant in the *S. torvum* cultivar / number of eggs per plant on the eggplant cv. Cristal).

4.4 Discussion

This study demonstrates for the first time that two consecutive crops of *S. torvum* in the same plots do not select for virulence in *M. incognita* but has an infective and reproductive fitness cost for the nematode in the susceptible eggplant. The resistance of *S. torvum* seems to be more stable than other *R*-genes in fruiting solanaceous crops such as tomato and pepper. In tomato, the selection for virulence to the *Mi1.2* gene can be acquired progressively crop by crop of resistant tomato cultivars (Giné and Sorribas 2017) or rootstocks (Verdejo-Lucas et al. 2009), or suddenly just after one tomato crop grafted onto the resistant rootstock cv. Aligator (Expósito et al. 2019). Regarding pepper, the selection for virulence to the *Me3* gene has been reported after two consecutive pepper crops grafted onto the rootstock cv. Atlante (Ros-Ibáñez et al. 2014). In relation to the *N* gene, virulence has been reported in the USA but without any information on the selection process (Thies 2011). Yang et al. (2014) consider that the entire disease resistance pathway is amplified in *S. torvum* compared with tomato and potato enhancing plant defense mechanisms and resistance durability.

The acquisition of virulence to a *R*-gene can have a fitness cost for the nematode in susceptible cultivars of the same plant species (Petrillo and Roberts 2005; Castagnone-Sereno et al. 2007; Djian-Caporalino et al. 2011; Expósito et al. 2019) after a minimum number of exposures to this *R*-gene. For example, three resistant tomato crops were needed to affect the infectivity, reproduction and fecundity of a partially virulent *M. incognita* subpopulation in susceptible tomato compared to the avirulent subpopulation (Expósito et al. 2019). Surprisingly, the results of this study revealed that the infective and reproductive fitness of the nematode decreased without having been selected for virulence after two years of repeated cultivation. The causes for this loss of fitness as well as the stability of this characteristic should be investigated. In a nematode field population a certain proportion of infective J2 can counteract the *S. torvum* resistance in a proportion that is maintained in the offspring irrespective of the plant resistance status in which they were originated, as it was observed in our experiment. Nonetheless, the proportion of the offspring originated in *S. torvum* has a fitness cost manifested in susceptible eggplant. This finding can have important consequences for managing *M. incognita* by agronomic methods since the nematode reproduction in susceptible eggplant decreased about 56%. Therefore, the use of different resistant sources in rotation with susceptible ones will decrease the risk of selecting virulent nematode populations. *Solanaceae* and *Cucurbitaceae* are the two most common botanical families used in rotation under protected cultivation (Moncada et al. 2013). In addition to the commercially available resistant tomato, pepper and eggplant cultivars and/or rootstocks, some other resistance sources in the *Cucurbitaceae* family such as *Cucumis metuliferus* and *Citrullus amarus* could be used as rootstocks in rotation schemes. Moreover, the resistance of *C. metuliferus*, *C. amarus* and *S. torvum* is also expressed against virulent populations to the *Mi-1.2* and *N* resistance genes (Expósito et al. 2018; García-Mendívil et al. 2019a). Plant resistance is an effective and economically profitable control method (Sorribas et al. 2005) that can be durable if it is used in a proper manner (Djian-Caporalino et al. 2011; Davies and Elling 2015). Among the proposed strategies to increase resistance durability, alternating only two resistance genes reduce virulence selection but not prevent it (Expósito et al. 2019). Including more resistance sources, some of them having a fitness cost for the nematode such as that in *S. torvum*, could prevent the selection for virulence to specific *R*-genes and could also reduce the infective and reproductive capability of the nematode in susceptible germplasm.

In relation to crop yield, significant differences between ungrafted and grafted eggplant were only detected at long cropping periods. An eggplant yield increase of 27% was recorded when cultivated during 9 months, but no differences were found at

cropping periods shorter than 6.5 months (Çürük et al. 2009; Gisbert et al. 2011; Moncada et al. 2013; Sabatino et al. 2013, 2019; Miceli et al. 2014; Bogoescu and Doltu 2015). In our study, grafted eggplant yielded a 110% more than the ungrafted when cropped during 8.3 months, but did not differ when cultivated during 4.5 months.

In summary, grafted eggplant onto *S. torvum* can yield significantly more in nematode infested soil depending on *Pi* and/or crop duration and is a valuable tool for managing the three tropical *Meloidogyne* spp. irrespective of its (a)virulence status to other *R*-genes in fruiting solanaceous crops; it reduces the infective and reproductive fitness of the nematode in susceptible after two consecutive crops without selecting for nematode virulence. However, special attention has to be taken in relation to variants of the nematode able to overcome resistance in *S. torvum*. Recently, the genotype A2-J of *M. arenaria* from Japan has been reported as virulent to *S. torvum*, but not the A2-O. Interestingly, the distribution area of the genotype A2-J overlaps with the cultivation area of eggplant (Uehara et al. 2017). Additional long term studies will be necessary to determine the resistance durability.

Acknowledgments

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R and AGL2017-89785-R, and CONACYT for providing PhD funding to H. A. García-Mendívil. Thank are also given to Manuel López-Gómez for providing the *M. javanica* MJLg and *M. incognita* MILgN populations, Manuel López-Gómez and Adriana Iglesias Culebras from Rijk Zwaan Iberica for providing the pepper seeds, Laura Mateo from Semillas Fitó for providing Brutus seeds, and to Sergi García, Alejandro Expósito, Anna Sanz and Miquel Masip for technical assistance. The authors have no conflict of interest to declare.

References

Ali, M., N. Matsuzoe, H. Okubo, and K. Fujieda. 1992. Resistance of Non-tuberous *Solanum* to Root-knot Nematode. *Horticultural science* 60 (4): 921–926. ISSN: 1880-358X. doi:10.2503/jjshs.60.921. http://joi.jlc.jst.go.jp/JST.Journalarchive/jjshs1925/60.921?from=CrossRef.

Araujo, M. T., M. J. Bassett, J. J. Augustine, and D. W. Dickson. 1982. Effects of the temperature and duration of the initial incubation period on resistance to *Meloidogyne incognita* in tomato. *Journal of nematology* 14 (3): 411.

Bogoescu, M. I. R. V., and M. Doltu. 2015. Effect of Grafting Eggplant (*Solanum melongena* L.) on its Selected Useful Characters. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture* 72, no. 2 (). ISSN: 1843-5394. doi:10.15835/buasvmcn-hort:11349. http://journals.usamvcluj.ro/index.php/horticulture/article/view/11349.

Castagnone-Sereno, P., M. Bongiovanni, and E. Wajnberg. 2007. Selection and parasite evolution: a reproductive fitness cost associated with virulence in the parthenogenetic nematode *Meloidogyne incognita*. *Evolutionary Ecology* 21, no. 2 (): 259–270. ISSN: 0269-7653. doi:10.1007/s10682-006-9003-5. http://link.springer.com/10.1007/s10682-006-9003-5.

Cortada, L., F. J. Sorribas, C. Ornat, I. Kaloshian, and S. Verdejo-Lucas. 2008. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathology* 57, no. 6 (): 1125–1135. ISSN: 00320862. doi:10.1111/j.1365-3059.2008.01906.x. http://doi.wiley.com/10.1111/j.1365-3059.2008.01906.x.

Çürük, S., H. Y. Dasgan, S. Mansuroğlu, Ş. Kurt, M. Mazmanoğlu, Ö. Antaklı, et al. 2009. Grafted eggplant yield, quality and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. *Pesquisa Agropecuária Brasileira* 44, no. 12 (): 1673–1681. ISSN: 0100-204X. doi:10.1590/S0100-204X2009001200017. http://www.scielo.br/scielo.php?script=sci%7B%5C_%7Darttext%7B%5C&%7Dpid=S0100-204X2009001200017%7B%5C&%7Dlng=en%7B%5C&%7Dtlng=en.

Daunay, M.-C. 2008. Eggplant. In *Vegetables II*, ed. by J. Prohens and F. Nuez, 163–220. New York: Springer. doi:10.1007/978-0-387-74110-9_5. http://link.springer.com/10.1007/978-0-387-74110-9%7B%5C_%7D5.

Davies, L. J., and A. A. Elling. 2015. Resistance genes against plant-parasitic nematodes: a durable control strategy? *Nematology* 17 (3): 249–263. ISSN: 1388-5545. doi:10.1163/15685411-00002877. https://brill.com/view/journals/nemy/17/3/article-p249%7B%5C_%7D1.xml.

Dhivya, R., A. Sadasakthi, and M. Sivakumar. 2014. Response of wild *Solanum* rootstocks to root-knot nematode (*Meloidogyne incognita* Kofoid and White). *International Journal of Plant Sciences* 9 (1): 117–122.

Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, et al. 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *European Journal of Plant Pathology* 131, no. 3 (): 431–440. ISSN: 0929-1873. doi:10.1007/s10658-011-9820-4. http://link.springer.com/10.1007/s10658-011-9820-4.

Expósito, A., S. García, A. Giné, N. Escudero, and F. J. Sorribas. 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato–melon rotation sequence. *Pest Management Science* 75, no. 7 (): 1902–1910. ISSN: 1526-498X. doi:10.1002/ps.5297. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.5297.

Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, M. B. Picó, et al. 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67, no. 5 (): 1161–1167. ISSN: 00320862. doi:10.1111/ppa.12815. http://doi.wiley.com/10.1111/ppa.12815.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Statistics Division. Visited on 09/04/2019. http://www.fao.org/faostat.

García-Mendívil, H. A., M. Munera, A. Giné, N. Escudero, M. B. Picó, C. Gisbert, et al. 2019a. Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. *Crop Protection* 119 (): 208–213. ISSN: 02612194. doi:10.1016/j.cropro.2019.02.005. https://linkinghub.elsevier.com/retrieve/pii/S0261219419300432.

García-Mendívil, H. A., N. Escudero, and F. J. Sorribas. 2019b. Host suitability of *Solanum torvum* cultivars to *Meloidogyne incognita* and *M. javanica* and population dynamics. *Plant Pathology* (): ppa.13036. ISSN: 13653059. doi:10.1111/

ppa.13036. https://onlinelibrary.wiley.com/doi/abs/10.1111/ppa.13036.

Giné, A., and F. J. Sorribas. 2017. Quantitative approach for the early detection of selection for virulence of *Meloidogyne incognita* on resistant tomato in plastic greenhouses. *Plant Pathology* 66, no. 8 (): 1338–1344. ISSN: 00320862. doi:10.1111/ppa.12679. http://doi.wiley.com/10.1111/ppa.12679.

Gisbert, C., J. Prohens, M. D. Raigón, J. R. Stommel, and F. Nuez. 2011. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. *Scientia Horticulturae* 128, no. 1 (): 14–22. ISSN: 03044238. doi:10.1016/j.scienta.2010.12.007. https://linkinghub.elsevier.com/retrieve/pii/S0304423810005431.

Greco, N., and M. Di Vito. 2009. 11 Population Dynamics and Damage Levels, ed. by R. Perry, M. Moens, and J. Starr, 246. CABI.

Hadisoeganda, W. W. 1982. Resistance of Tomato, Bean, Southern Pea, and Garden Pea Cultivars to Root-Knot Nematodes Based on Host Suitability. *Plant Disease* 66 (1): 145. ISSN: 0191-2917. doi:10.1094/PD-66-145. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1982Abstracts/PD%7B%5C_%7D66%7B%5C_%7D145.htm.

Hallmann, J., and B. H. Meressa. 2018. Nematode parasites of vegetables. In *Plant parasitic nematodes in subtropical and tropical agriculture*, ed. by R. Sikora, D. Coyne, J. Hallman, and P. Timper. Wallingford: CABI. doi:10.1079/9781786391247.0346.

Hussey, R. S., and K. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025–1028.

Miceli, A., L. Sabatino, A. Moncada, F. Vetrano, and F. D'Anna. 2014. Nursery and field evaluation of eggplant grafted onto unrooted cuttings of *Solanum torvum* Sw. *Scientia Horticulturae* 178 (): 203–210. ISSN: 03044238. doi:10.1016/j.scienta.2014.08.025. https://linkinghub.elsevier.com/retrieve/pii/S0304423814004774.

Moncada, A., A. Miceli, F. Vetrano, V. Mineo, D. Planeta, and F. D'Anna. 2013. Effect of grafting on yield and quality of eggplant (*Solanum melongena* L.) *Scientia Horticulturae* 149 (): 108–114. ISSN: 03044238. doi:10.1016/j.scienta.2012.06.015. https://linkinghub.elsevier.com/retrieve/pii/S0304423812002762.

Nyczepir, A. P., and S. H. Thomas. 2009. Current and future management strategies in intensive crop production systems. In *Root-knot nematodes*, ed. by R. Perry, M. Moens, and J. Starr, 412–443. Wallingford: CABI. doi:10.1079/9781845934927.0412. http://www.cabi.org/cabebooks/ebook/20093330198.

Öçal, S., T. Özalp, and Z. Devran. 2018. Reaction of wild eggplant *Solanum torvum* to different species of root-knot nematodes from Turkey. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/s41348-018-0167-3.

Oda, M. 2002. Grafting of vegetable crops. *Scientific Report of Agricultural and Biological Science*. https://www.cabdirect.org/cabdirect/abstract/20023124609.

Petrillo, M. D., and P. A. Roberts. 2005. Fitness of Virulent *Meloidogyne incognita* Isolates on Susceptible and Resistant Cowpea. *Journal of nematology* 37, no. 4 (): 457–66. ISSN: 0022-300X. http://www.ncbi.nlm.nih.gov/pubmed/19262891%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2620988.

Rahman, M. A., M. A. Rashid, M. A. Salam, M. A. T. Masud, A. S. M. H. Masum, and M. M. Hossain. 2002. Performance of Some Grafted Eggplant Genotypes on Wild *Solanum* Root Stocks Against Root-Knot Nematode. *Journal of Biological Sciences* 2, no. 7 (): 446–448. ISSN: 17273048. doi:10.3923/jbs.2002.446.448. http://www.scialert.net/abstract/?doi=jbs.2002.446.448.

Ranil, R. H., H. M. Niran, M. Plazas, R. M. Fonseka, H. H. Fonseka, S. Vilanova, et al. 2015. Improving seed germination of the eggplant rootstock *Solanum torvum* by testing multiple factors using an orthogonal array design. *Scientia Horticulturae*. ISSN: 03044238. doi:10.1016/j.scienta.2015.07.030.

Roberts, P. A. 2009. Concepts and consequences of resistance. In *Plant resistance to parasitic nematodes*, ed. by J. Starr, R. Cook, and J. Bridge. Wallingford: CABI. doi:10.1079/9780851994666.0023.

Ros-Ibáñez, C., L. Robertson, M. d. C. Martínez-Lluch, A. Cano-García, and A. Lacasa-Plasencia. 2014. Development of virulence to *Meloidogyne incognita* on resistant pepper rootstocks. *Spanish Journal of Agricultural Research*. ISSN: 1695971X. doi:10.5424/sjar/2014121-4340.

Sabatino, L., E. Palazzolo, and F. D'Anna. 2013. Grafting suitability of Sicilian eggplant ecotypes onto *Solanum torvum*: Fruit composition, production and phenology. *Journal of Food, Agriculture and Environment*. ISSN: 14590263.

Sabatino, Iapichino, Rotino, Palazzolo, Mennella, and D'Anna. 2019. *Solanum aethiopicum* gr. gilo and Its Interspecific Hybrid with *S. melongena* as Alternative Rootstocks for Eggplant: Effects on Vigor, Yield, and Fruit Physicochemical Properties of Cultivar 'Scarlatti'. *Agronomy*. doi:10.3390/agronomy9050223.

Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano, and J. Valero. 2005. Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology* 111, no. 1 (): 29–38. ISSN: 0929-1873. doi:10.1007/s10658-004-1982-x. http://link.springer.com/10.1007/s10658-004-1982-x.

Thies, J. A. 2011. Virulence of *Meloidogyne incognita* to expression of *N* gene in pepper. *Journal of nematology* 43, no. 2 (): 90–4. ISSN: 2640-396X. http://www.ncbi.nlm.nih.gov/pubmed/22791917%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3380459.

Thies, J. A., S. Buckner, M. Horry, R. Hassell, and A. Levi. 2015. Influence of *Citrullus lanatus* var. *citroides* Rootstocks and Their F1 Hybrids on Yield and Response to Root-knot Nematode, *Meloidogyne incognita*, in Grafted Watermelon. *HortScience* 50, no. 1 (): 9–12. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.9. https://journals.ashs.org/view/journals/hortsci/50/1/article-p9.xml.

Tzortzakakis, E. A., F. A. Bletsos, and A. D. Avgelis. 2006. Evaluation of *Solanum* rootstock accessions for control of root-knot nematodes and tobamoviruses. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/BF03356178.

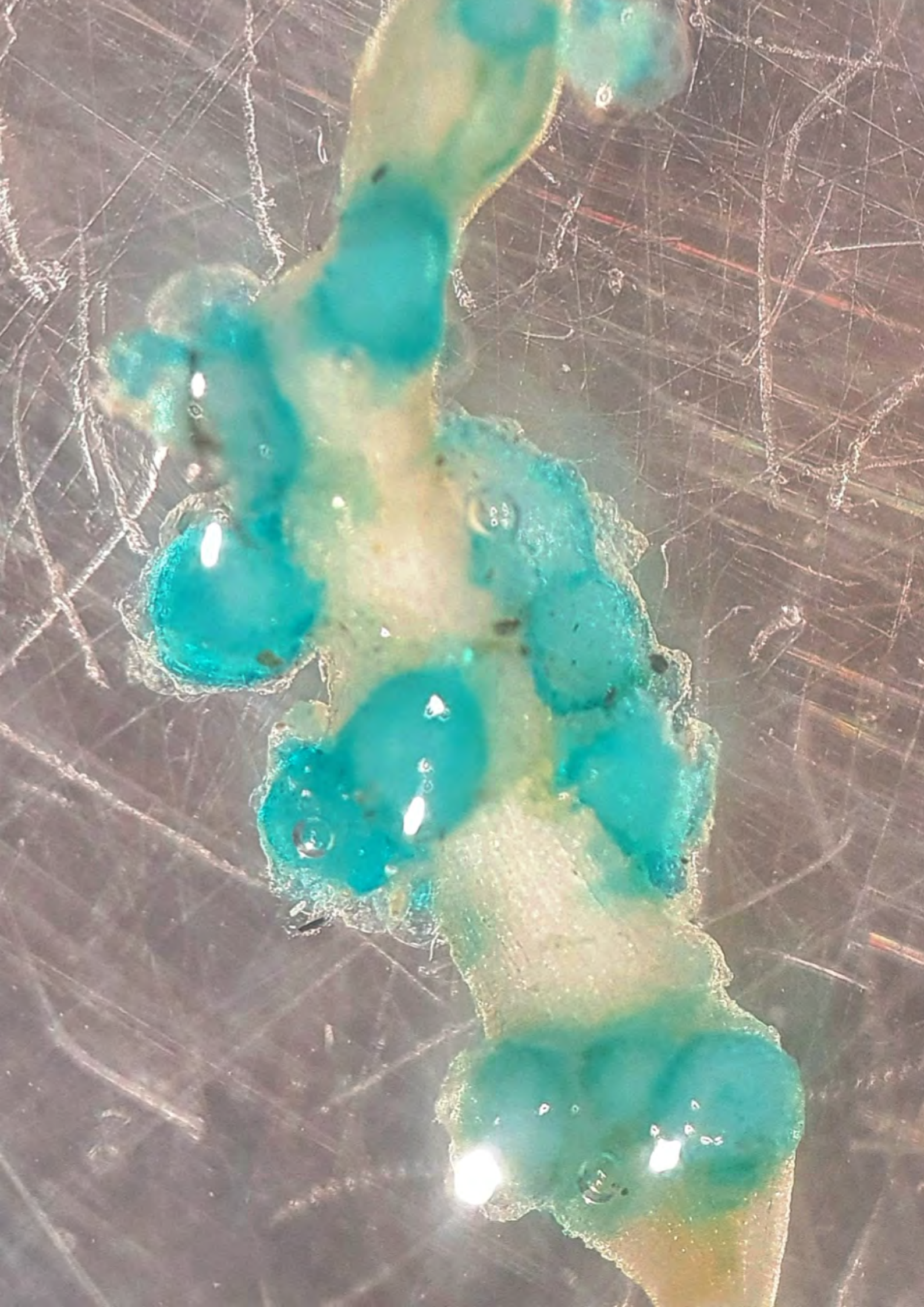
Uehara, T., Y. Tateishi, Y. Kadota, and H. Iwahori. 2017. Differences in parasitism of *Meloidogyne incognita* and two genotypes of *M. arenaria* on *Solanum torvum* in Japan. *Journal of Phytopathology*. ISSN: 14390434. doi:10.1111/jph.12594.

Verdejo-Lucas, S., L. Cortada, F. J. Sorribas, and C. Ornat. 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of mi resistance gene tomato rootstocks under field conditions. *Plant Pathology*. ISSN: 00320862. doi:10.1111/j.1365-3059.2009.02089.x.

Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, no. 1 (): 25–38. ISSN: 0003-4746. doi:10.1111/j.1744-7348.1965.tb07864.x. http://doi.wiley.com/10.1111/j.1744-7348.1965.tb07864.x.

Yang, X., Y. F. Cheng, C. Deng, Y. Ma, Z. W. Wang, X. H. Chen, et al. 2014. Comparative transcriptome analysis of eggplant (*Solanum melongena* L.) and turkey berry (*Solanum torvum* Sw.): Phylogenomics and disease resistance analysis. *BMC Genomics*. ISSN: 14712164. doi:10.1186/1471-2164-15-412.

Zeck, W. M. 1971. Rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschutz nachrichten*, no. 24: 141–144. ISSN: 0079-1342.



General discussion

The *Citrullus amarus* accessions and the *Solanum torvum* cultivars studied in this thesis demonstrated to be resistant to the majority of *Meloidogyne* populations of the three most widespread species occurring in Spain. Particularly interesting, performed as resistant to virulent isolates to the *Mi1.2* and *N* resistance genes of tomato and pepper, respectively. Reports about virulent populations are increasing (Ornat et al. 2007; Verdejo-Lucas et al. 2009; Thies 2011; Verdejo-Lucas et al. 2012; Ros-Ibáñez et al. 2014; Uehara et al. 2017b; Öçal et al. 2018). Then, the use of the studied rootstocks in growing areas with such virulent populations could be remarkable help to deal with this issue.

As population dynamic parameters pointed out, the response of the *C. amarus* and *S. torvum* rootstocks to increasing nematode densities also revealed its exceptionally poor hosts status. Interestingly, both were less suitable host for *M. incognita* than for *M. javanica*. Previous research also found *C. amarus* accessions and *S. torvum* rootstocks to be resistant to *M. incognita* (Rahman et al. 2002; Huitrón et al. 2007; Dhivya et al. 2014; Thies et al. 2008; 2009; 2015; 2015; 2015; Uehara et al. 2017a), and for *S. torvum*, a wide range of responses to *M. javanica* (Daunay 2008; Tzortzakakis et al. 2006; Öçal et al. 2018). Interestingly, in contrast with the cucurbit species studied, an apparent decrease in root fibrosity seems to be associated with increases in a and M values, and relatively low E values compared to M. Besides lower values of a and M.

C. amarus and *S. torvum* showed the same ability to maintain its resistance levels after being cultivated for two consecutive growing seasons in the same plot. Previous studies found evidence of partial or complete loss of resistance in tomato or pepper germplasm carrying the *Mi1.2*, *Me3* or *N* *R*-genes in the same number of seasons (Castagnone-Sereno et al. 2007; Verdejo-Lucas et al. 2009; Thies 2011; Giné and Sorribas 2017; Expósito et al. 2019). Then, it is an indicator of durability of resistance. In addition, both rootstocks studied had an infective and reproductive fitness cost for the nematode. That is, the reproduction of the nematode population coming from grafted watermelon or eggplant was less than 50% when inoculated in a susceptible watermelon or eggplant, compared to the reproduction of a population coming from a susceptible host. This acquisition of fitness cost in a susceptible cultivar have been previously reported after a minimum amount of exposures to the same *R*-gene (Petrillo and Roberts 2005; Castagnone-Sereno et al. 2007; Djian-Caporalino et al. 2011; Expósito et al. 2019), but only at the expense of virulence selection. The effect of this fitness cost on rotation schemes including resistant and susceptible germplasm should be studied in field conditions.

Tolerance to the nematode population densities evaluated in this thesis was also found in rootstocks of both families, as no difference in dry top weight were found. This was also evidenced in the plastic greenhouse experiments, where rootstock-scion compatibility was manifested in yield increments, more than double in some cases, when compared with ungrafted plants. In accordance, previous studies also found increments, or at least no differences, in yield of both watermelon (Thies et al. 2015a; 2015b) and eggplant (Çürük et al. 2009;

Gisbert et al. 2011; Moncada et al. 2013; Sabatino et al. 2013, 2019; Miceli et al. 2014; Bogoescu and Doltu 2015). Another remark on this subject, was the work done by the Dr Maria Belén Picó and Dr. Carmina Gisbert, described in chapter 1, that showed null effect of the CI67 accession over the watermelon fruit quality, which was in accordance with Fredes et al. (2017), that also found similar results when grafting the F1 hybrid cv. Oneida onto CI67. Although this thesis have not evaluated eggplant fruit quality, previous research also found no negative effects on yield and quality, however, it may increase the size of fruit and also reduce its lightness and saturation of color, thus, more research on the effect of grafting on fruit quality needs to be done to assure the standards demanded by the market.

In summary, a) the poor host status to a wide range of populations, included some virulent to most cultivated *R*-genes, b) the absent selection for virulence after two cropping seasons in the same plot, c) the fitness cost acquired by the nematode population, and d) the effect on crop yield, makes evident the usefulness of both, *C. amarus* and *S. torvum*, to be employed in rotation schemes. Previous studies found that the inclusion of only two genetic sources of resistance in a rotation scheme are not enough to avoid virulence selection (Expósito et al. 2019), however, it does reduce its levels. Thus, including these two more sources of resistance could be a solution to avoid virulence selection and improving crop yield. Some experiments to assess the validity of this hypothesis are currently ongoing. That should be studied in the future.

Conclusions

Conclusions on *C. amarus* accessions:

I. The *C. amarus* accessions BGV0005164 and BGV0005167 are resistant to *Meloidogyne* isolates belonging to the three most widespread RKN species, including one virulent to the *Mi1.2* resistance gene.

II. *C. amarus* accessions are less suitable host for *M. incognita* than for *M. javanica*.

III. Watermelon cv. Sugar baby and both *C. amarus* accessions are tolerant to *M. incognita* and *M. javanica*.

IV. Cropping both *C. amarus* accessions two consecutive growing seasons in the same plots are not enough time to reduce its resistance levels nor to select for virulence in *M. incognita*, but it has an infective and reproductive fitness cost for the nematode in watermelon.

V. Grafting onto both *C. amarus* accessions improves watermelon yield.

Conclusions on *S. torvum* rootstocks:

VI. *S. torvum* rootstocks are resistant to several *M. incognita* and *M. javanica* isolates, including two virulent isolates to the *Mi1.2* gene and, for first time reported, *N* resistance gene.

VII. *S. torvum* rootstocks are less suitable host for *M. incognita* than for *M. javanica*.

VIII. The cropping season influence the population growth rate of the nematode in *S. torvum* rootstocks and, to a higher degree, in eggplant.

IX. Cultivar Brutus confers tolerance to *M. javanica*.

X. Cropping *S. torvum* two consecutive growing seasons in the same plots do not select for virulence in *M. incognita* but has an infective and reproductive fitness cost for the nematode in eggplant.

XI. Grafting onto *S. torvum* improves eggplant yield when cultivated for long periods (8.3 months), but does not differ when cultivated in shorter periods (4.5 months).

General conclusion

XII. Rootstocks from both families present particularly useful characteristics that suggest its convenience to be employed in rotation scheme as supplementary genetic source of resistance in order to increase its diversity.

References

Abad, P., B. Favery, M.-N. Rosso, and P. Castagnone-Sereno. 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology* 4, no. 4 (): 217–224. ISSN: 1464-6722. doi:10.1046/j.1364-3703.2003.00170.x. http://doi.wiley.com/10.1046/j.1364-3703.2003.00170.x.

Ali, M., N. Matsuzoe, H. Okubo, and K. Fujieda. 1992. Resistance of Non-tuberous *Solanum* to Root-knot Nematode. *Horticultural science* 60 (4): 921–926. ISSN: 1880-358X. doi:10.2503/jjshs.60.921. http://joi.jlc.jst.go.jp/JST.Journalarchive/jjshs1925/60.921?from=CrossRef.

Araujo, M. T., M. J. Bassett, J. J. Augustine, and D. W. Dickson. 1982. Effects of the temperature and duration of the initial incubation period on resistance to *Meloidogyne incognita* in tomato. *Journal of nematology* 14 (3): 411.

Bletsos, F., C. Thanassoulopoulos, and D. Roupakias. 2003. Effect of Grafting on Growth, Yield, and Verticillium Wilt of Eggplant. *HortScience* 38, no. 2 (): 183–186. ISSN: 0018-5345. doi:10.21273/HORTSCI.38.2.183. https://journals.ashs.org/view/journals/hortsci/38/2/article-p183.xml.

Bogoescu, M. I. R. V., and M. Doltu. 2015. Effect of Grafting Eggplant (*Solanum melongena* L.) on its Selected Useful Characters. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture* 72, no. 2 (). ISSN: 1843-5394. doi:10.15835/buasvmcn-hort:11349. http://journals.usamvcluj.ro/index.php/horticulture/article/view/11349.

Boiteux, L. S., and J. M. Charchar. 1996. Genetic resistance to root-knot nematode (*Meloidogyne javanica*) in eggplant (*Solanum melongena*). *Plant Breeding* 115, no. 3 (): 198–200. ISSN: 0179-9541. doi:10.1111/j.1439-0523.1996.tb00902.x. http://doi.wiley.com/10.1111/j.1439-0523.1996.tb00902.x.

Castagnone-Sereno, P., M. Bongiovanni, and E. Wajnberg. 2007. Selection and parasite evolution: a reproductive fitness cost associated with virulence in the parthenogenetic nematode *Meloidogyne incognita*. *Evolutionary Ecology* 21, no. 2 (): 259–270. ISSN: 0269-7653. doi:10.1007/s10682-006-9003-5. http://link.springer.com/10.1007/s10682-006-9003-5.

Chapman, M. A. 2019. Introduction: The Importance of Eggplant, ed. by M. A. Chapman, 1–10. Springer. ISBN: 9783319992082. doi:10.1007/978-3-319-99208-2_1. http://link.springer.com/10.1007/978-3-319-99208-2%7B%5C_%7D1.

Chomicki, G., and S. S. Renner. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. *New Phytologist* 205, no. 2 (): 526–532. ISSN: 0028646X. doi:10.1111/nph.13163. http://doi.wiley.com/10.1111/nph.13163.

Cohen, R., Y. Burger, C. Horev, A. Koren, and M. Edelstein. 2007. Introducing Grafted Cucurbits to Modern Agriculture: The Israeli Experience. *Plant Disease* 91, no. 8 (): 916–923. ISSN: 0191-2917. doi:10.1094/PDIS-91-8-0916. http://apsjournals.apsnet.org/doi/10.1094/PDIS-91-8-0916.

Cortada, L., F. J. Sorribas, C. Ornat, I. Kaloshian, and S. Verdejo-Lucas. 2008. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathology* 57, no. 6 (): 1125–1135. ISSN: 00320862. doi:10.1111/j.1365-3059.2008.01906.x. http://doi.wiley.com/10.1111/j.1365-3059.2008.01906.x.

Çürük, S., H. Y. Dasgan, S. Mansuroğlu, Ş. Kurt, M. Mazmanoğlu, Ö. Antaklı, et al. 2009. Grafted eggplant yield, quality and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. *Pesquisa Agropecuária Brasileira* 44, no. 12 (): 1673–1681. ISSN: 0100-204X. doi:10.1590/S0100-204X2009001200017. http://www.scielo.br/scielo.php?script=sci%7B%5C_%7Darttext%7B%5C&%7Dpid=S0100-204X2009001200017%7B%5C&%7Dlng=en%7B%5C&%7Dtlng=en.

Dane, F., and P. Lang. 2004. Sequence variation at cpDNA regions of watermelon and related wild species: implications for the evolution of *Citrullus haplotypes*. *American Journal of Botany* 91, no. 11 (): 1922–1929. ISSN: 0002-9122. doi:10.3732/ajb.91.11.1922. http://doi.wiley.com/10.3732/ajb.91.11.1922.

Daunay, M.-C. 2008. Eggplant. In *Vegetables II*, ed. by J. Prohens and F. Nuez, 163–220. New York: Springer. doi:10.1007/978-0-387-74110-9_5. http://link.springer.com/10.1007/978-0-387-74110-9%7B%5C_%7D5.

Daunay, M.-C., and A. Dalmasso. 1985. Multiplication de *Meloidogyne javanica*, *M. incognita* et *M. arenaria* sur divers Solarium (’). *Revue Nématol* 8 (1): 31–34.

Davis, A. R., P. Perkins-Veazie, Y. Sakata, S. López-Galarza, J. V. Maroto, S.-G. Lee, et al. 2008. Cucurbit Grafting. *Critical Reviews in Plant Sciences* 27, no. 1 (): 50–74. ISSN: 0735-2689. doi:10.1080/07352680802053940. http://www.tandfonline.com/doi/abs/10.1080/07352680802053940.

Dhivya, R., A. Sadasakthi, and M. Sivakumar. 2014. Response of wild *Solanum* rootstocks to root-knot nematode (*Meloidogyne incognita* Kofoid and White). *International Journal of Plant Sciences* 9 (1): 117–122.

Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, et al. 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *European Journal of Plant Pathology* 131, no. 3 (): 431–440. ISSN: 0929-1873. doi:10.1007/s10658-011-9820-4. http://link.springer.com/10.1007/s10658-011-9820-4.

Djian-Caporalino, C., A. Palloix, A. Fazari, N. Marteu, A. Barbary, P. Abad, et al. 2014. Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. *BMC plant biology* 14 (1): 53.

Expósito, A., S. García, A. Giné, N. Escudero, and F. J. Sorribas. 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato–melon rotation sequence. *Pest Management Science* 75, no. 7 (): 1902–1910. ISSN: 1526-498X. doi:10.1002/ps.5297. https://onlinelibrary.wiley.com/doi/abs/10.1002/ps.5297.

Expósito, A., M. Munera, A. Giné, M. López-Gómez, A. Cáceres, M. B. Picó, et al. 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67, no. 5 (): 1161–1167. ISSN: 00320862. doi:10.1111/ppa.12815. http://doi.wiley.com/10.1111/ppa.12815.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Statistics Division. Visited on 09/04/2019. http://www.fao.org/faostat.

Fassuliotis, G. 1970. Resistance of *Cucumis* spp. to the root-knot nematode, *Meloidogyne incognita* acrita. *Journal of Nematology* 2 (2): 174.

Ferris, H., and S. Van Gundy. 1979. *Meloidogyne* ecology and host interrelationships. *Meloidogyne ecology and host interrelationships.*: 205–230.

Ferris, H. 1985. Population assessment and management strategies for plant-parasitic nematodes. *Agriculture, Ecosystems & Environment* 12, no. 4 (): 285–299. ISSN: 01678809. doi:10.1016/0167-8809(85)90003-9. https://linkinghub.elsevier.com/retrieve/pii/0167880985900039.

Fredes, A., S. Roselló, J. Beltrán, J. Cebolla-Cornejo, A. Pérez-de-Castro, C. Gisbert, et al. 2017. Fruit quality assessment of watermelons grafted onto citron melon rootstock. *Journal of the Science of Food and Agriculture* 97, no. 5 (): 1646–1655. ISSN: 00225142. doi:10.1002/jsfa.7915. http://doi.wiley.com/10.1002/jsfa.7915.

Giné, A., and F. J. Sorribas. 2017. Quantitative approach for the early detection of selection for virulence of *Meloidogyne incognita* on resistant tomato in plastic greenhouses. *Plant Pathology* 66, no. 8 (): 1338–1344. ISSN: 00320862. doi:10.1111/ppa.12679. http://doi.wiley.com/10.1111/ppa.12679.

Giné, A., C. González, L. Serrano, and F. J. Sorribas. 2017. Population dynamics of *Meloidogyne incognita* on cucumber grafted onto the Cucurbita hybrid RS841 or ungrafted and yield losses under protected cultivation. *European Journal of Plant Pathology* 148, no. 4 (): 795–805. ISSN: 0929-1873. doi:10.1007/s10658-016-1135-z. http://link.springer.com/10.1007/s10658-016-1135-z.

Giné, A., M. López-Gómez, M. D. Vela, C. Ornat, M. Talavera, S. Verdejo-Lucas, et al. 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology* 63, no. 6 (): 1446–1453. ISSN: 00320862. doi:10.1111/ppa.12217. http://doi.wiley.com/10.1111/ppa.12217.

Gisbert, C., J. Prohens, M. D. Raigón, J. R. Stommel, and F. Nuez. 2011. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. *Scientia Horticulturae* 128, no. 1 (): 14–22. ISSN: 03044238. doi:10.1016/j.scienta.2010.12.007. https://linkinghub.elsevier.com/retrieve/pii/S0304423810005431.

Greco, N., and M. Di Vito. 2009. 11 Population Dynamics and Damage Levels, ed. by R. Perry, M. Moens, and J. Starr, 246. CABI.

Guner, N., and T. C. Wehner. 2008. Overview of Potyvirus resistance in watermelon. *Journal of Heredity*. https://w3.avignon.inra.fr/dspace/handle/2174/245.

Guo, S., J. Zhang, H. Sun, J. Salse, W. J. Lucas, H. Zhang, et al. 2013. The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nature Genetics* 45, no. 1 (): 51–58. ISSN: 1061-4036. doi:10.1038/ng.

2470. http://dx.doi.org/10.1038/ng.2470%20http://www.nature.com/articles/ng.2470.

Gusmini, G., R. Song, and T. C. Wehner. 2005. New Sources of Resistance to Gummy Stem Blight in Watermelon. *Crop Science* 45 (2): 582. ISSN: 1435-0653. doi:10.2135/cropsci2005.0582. https://www.crops.org/publications/cs/abstracts/45/2/0582.

Hadisoeganda, W. W. 1982. Resistance of Tomato, Bean, Southern Pea, and Garden Pea Cultivars to Root-Knot Nematodes Based on Host Suitability. *Plant Disease* 66 (1): 145. ISSN: 0191-2917. doi:10.1094/PD-66-145. http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1982Abstracts/PD%7B%5C_%7D66%7B%5C_%7D145.htm.

Hallmann, J., and B. H. Meressa. 2018. Nematode parasites of vegetables. In *Plant parasitic nematodes in subtropical and tropical agriculture*, ed. by R. Sikora, D. Coyne, J. Hallman, and P. Timper. Wallingford: CABI. doi:10.1079/9781786391247.0346.

Hara, T., S. Kusakari, K. Abe, K. Yamada, and Y. Momota. 1983. Studies on damage by continuous cropping of eggplants growing in walk-in plastic clad tunnels, 1: The occurrence of damage by continuous cropping. *Bulletin of the Osaka Agricultural Research Center (Japan)*.

Huitrón, M. V., M. Diaz, F. Diánez, and F. Camacho. 2007. The effect of various rootstocks on triploid watermelon yield and quality. *Journal of Food, Agriculture and Environment*. ISSN: 14590263. https://pubag.nal.usda.gov/catalog/723527.

Hwang, J. H., S. G. Ahn, J. Y. Oh, Y. W. Choi, J. S. Kang, and Y. H. Park. 2011. Functional characterization of watermelon (*Citrullus lanatus* L.) EST–SSR by gel electrophoresis and high resolution melting analysis. *Scientia Horticulturae* 130, no. 4 (): 715–724. ISSN: 03044238. doi:10.1016/j.scienta.2011.08.014. https://linkinghub.elsevier.com/retrieve/pii/S0304423811004201.

Jones, J. T., A. Haegeman, E. G. J. Danchin, H. S. Gaur, J. Helder, M. G. K. Jones, et al. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*. ISSN: 14646722. doi:10.1111/mpp.12057.

Knapp, S., M. S. Vorontsova, and J. Prohens. 2013. Wild Relatives of the Eggplant (*Solanum melongena* L.: *Solanaceae*): New Understanding of Species Names in a Complex Group. Ed. by D. Q. Fuller. *PLoS ONE* 8, no. 2 (): e57039. ISSN: 1932-6203. doi:10.1371/journal.pone.0057039. http://dx.plos.org/10.1371/journal.pone.0057039.

Kokalis-Burelle, N., and E. N. Rosskopf. 2011. Microplot Evaluation of Rootstocks for Control of *Meloidogyne incognita* on Grafted Tomato, Muskmelon, and Watermelon. *Journal of nematology* 43, **numbers** 3-4 (): 166–71. ISSN: 0022-300X. http://www.ncbi.nlm.nih.gov/pubmed/23431109%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3547354.

Kumar, S., N. Bharti, and S. N. Saravaiya. 2018. Vegetable Grafting: A Surgical Approach to combat biotic and abiotic stresses: A review. *Agricultural Reviews*. ISSN: 0253-1496. doi:10.18805/ag.r-1711.

Kyriacou, M. C., Y. Rouphael, G. Colla, R. Zrenner, and D. Schwarz. 2017. Vegetable Grafting: The Implications of a Growing Agronomic Imperative for Vegetable Fruit Quality and Nutritive Value. *Frontiers in Plant Science* 8, no. May (): 1–23. ISSN: 1664-462X. doi:10.3389/fpls.2017.00741. http://journal.frontiersin.org/article/10.3389/fpls.2017.00741/full.

Lambel, S., B. Lanini, E. Vivoda, J. Fauve, W. Patrick Wechter, K. R. Harris-Shultz, et al. 2014. A major QTL associated with *Fusarium oxysporum* race 1 resistance identified in genetic populations derived from closely related watermelon lines using selective genotyping and genotyping-by-sequencing for SNP discovery. *Theoretical and Applied Genetics* 127, no. 10 (): 2105–2115. ISSN: 0040-5752. doi:10.1007/s00122-014-2363-2. http://link.springer.com/10.1007/s00122-014-2363-2.

Levi, A., C. E. Thomas, A. P. Keinath, and T. C. Wehner. 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genetic Resources and Crop Evolution* 48 (6): 559–566. ISSN: 09259864. doi:10.1023/A:1013888418442.

Levi, A., C. E. Thomas, T. C. Wehner, and X. Zhang. 2001b. Low Genetic Diversity Indicates the Need to Broaden the Genetic Base of Cultivated Watermelon. *HortScience* 36, no. 6 (): 1096–1101. ISSN: 0018-5345. doi:10.21273/HORTSCI.36.6.1096. https://journals.ashs.org/view/journals/hortsci/36/6/article-p1096.xml.

López-Gómez, M., E. Flor-Peregrín, M. Talavera, F. J. Sorribas, and S. Verdejo-Lucas. 2015. Population dynamics of *Meloidogyne javanica* and its relationship with the leaf chlorophyll content in zucchini. *Crop Protection* 70 (): 8–14. ISSN: 02612194. doi:10.1016/j.cropro.2014.12.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219414003871.

López-Gómez, M., A. Giné, M. D. Vela, C. Ornat, F. J. Sorribas, M. Talavera, et al. 2014. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. *Annals of Applied Biology* 165, no. 3 (): 466–473. ISSN: 00034746. doi:10.

1111/aab.12154. http://doi.wiley.com/10.1111/aab.12154.

López-Gómez, M., M. Talavera, and S. Verdejo-Lucas. 2016. Differential reproduction of *Meloidogyne incognita* and *M. javanica* in watermelon cultivars and cucurbit rootstocks. *Plant Pathology* 65, no. 1 (): 145–153. ISSN: 13653059. doi:10.1111/ppa.12394. http://doi.wiley.com/10.1111/ppa.12394.

MAPA. 2019. Información subsectorial. Producciones agricolas. *Ministerio de Agricultura Pesca y Alimentacion*. Visited on 09/04/2019. https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/melonysandiacampana2019abril-julio%7B%5C_%7Dtcm30-511671.pdf.

Maynard, D., and D. Maynard. 2000. Cucumbers, melons, and watermelons. In *The Cambridge world history of food*, ed. by K. Kiple and K. Ornelas, 298–313. Cambridge: Cambridge University Press.

McCreight, J. D., J. E. Staub, T. C. Wehner, and N. P. Dhillon. 2013. Gone Global: Familiar and Exotic Cucurbits Have Asian Origins. *HortScience* 48, no. 9 (): 1078–1089. ISSN: 0018-5345. doi:10.21273/HORTSCI.48.9.1078. https://journals.ashs.org/view/journals/hortsci/48/9/article-p1078.xml.

Meyer, R. S., B. D. Whitaker, D. P. Little, S.-B. Wu, E. J. Kennelly, C.-L. Long, et al. 2015. Parallel reductions in phenolic constituents resulting from the domestication of eggplant. *Phytochemistry* 115 (): 194–206. ISSN: 00319422. doi:10.1016/j.phytochem.2015.02.006. https://linkinghub.elsevier.com/retrieve/pii/S0031942215000539.

Miceli, A., L. Sabatino, A. Moncada, F. Vetrano, and F. D'Anna. 2014. Nursery and field evaluation of eggplant grafted onto unrooted cuttings of *Solanum torvum* Sw. *Scientia Horticulturae* 178 (): 203–210. ISSN: 03044238. doi:10.1016/j.scienta.2014.08.025. https://linkinghub.elsevier.com/retrieve/pii/S0304423814004774.

Miguel, A., J. V. Maroto, A. San Bautista, C. Baixauli, V. Cebolla, B. Pascual, et al. 2004. The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. *Scientia Horticulturae* 103, no. 1 (): 9–17. ISSN: 03044238. doi:10.1016/j.scienta.2004.04.007. https://linkinghub.elsevier.com/retrieve/pii/S030442380400086X.

Mo, Y., R. Yang, L. Liu, X. Gu, X. Yang, Y. Wang, et al. 2016. Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. *Plant Growth Regulation* 79, no. 2 (): 229–241. ISSN: 0167-6903. doi:10.1007/s10725-015-0128-9. http://link.springer.com/10.1007/s10725-015-0128-9.

Moens, M., R. N. Perry, J. L. Starr, et al. 2009. *Meloidogyne* species–a diverse group of novel and important plant parasites. *Root-knot nematodes* 1:483.

Moncada, A., A. Miceli, F. Vetrano, V. Mineo, D. Planeta, and F. D'Anna. 2013. Effect of grafting on yield and quality of eggplant (*Solanum melongena* L.) *Scientia Horticulturae* 149 (): 108–114. ISSN: 03044238. doi:10.1016/j.scienta.2012.06.015. https://linkinghub.elsevier.com/retrieve/pii/S0304423812002762.

Ngwepe, R. M., J. Mashilo, and H. Shimelis. 2019. Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. *Genetic Resources and Crop Evolution* 66, no. 3 (): 735–758. ISSN: 0925-9864. doi:10.1007/s10722-018-0724-4. https://doi.org/10.1007/s10722-018-0724-4%20http://link.springer.com/10.1007/s10722-018-0724-4.

Nimmakayala, P., A. Levi, L. Abburi, V. Abburi, Y. R. Tomason, T. Saminathan, et al. 2014. Single nucleotide polymorphisms generated by genotyping by sequencing to characterize genome-wide diversity, linkage disequilibrium, and selective sweeps in cultivated watermelon. *BMC Genomics* 15 (1): 767. ISSN: 1471-2164. doi:10.1186/1471-2164-15-767. http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-15-767.

Nyczepir, A. P., and S. H. Thomas. 2009. Current and future management strategies in intensive crop production systems. In *Root-knot nematodes*, ed. by R. Perry, M. Moens, and J. Starr, 412–443. Wallingford: CABI. doi:10.1079/9781845934927.0412. http://www.cabi.org/cabebooks/ebook/20093330198.

Öçal, S., T. Özalp, and Z. Devran. 2018. Reaction of wild eggplant *Solanum torvum* to different species of root-knot nematodes from Turkey. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/s41348-018-0167-3.

Oda, M. 2002. Grafting of vegetable crops. *Scientific Report of Agricultural and Biological Science*. https://www.cabdirect.org/cabdirect/abstract/20023124609.

Oda, M., and J.-M. Lee. 2003. Grafting of Herbaceous Vegetable and Ornamental Crops. In *Horticultural Reviews*, 61–124. Oxford, UK: John Wiley & Sons, Inc. doi:10.1002/9780470650851.ch2. http://doi.wiley.com/10.1002/9780470650851.ch2.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 2007. A Population of *Meloidogyne javanica* in Spain Virulent to the *Mi* Resistance Gene in Tomato. *Plant Disease* 85, no. 3 (): 271–276. ISSN: 0191-2917. doi:10.1094/PDIS.2001.85.3.271. http://apsjournals.apsnet.org/doi/10.1094/PDIS.2001.85.3.271.

Ornat, C., S. Verdejo-Lucas, and F. J. Sorribas. 1997. Effect of the previous crop on population densities of *Meloidogyne javanica* and yield of cucumber. *Nematropica*. ISSN: 0099-5444.

Petrillo, M. D., and P. A. Roberts. 2005. Fitness of Virulent *Meloidogyne incognita* Isolates on Susceptible and Resistant Cowpea. *Journal of nematology* 37, no. 4 (): 457–66. ISSN: 0022-300X. http://www.ncbi.nlm.nih.gov/pubmed/19262891%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2620988.

Rahman, M. A., M. A. Rashid, M. A. Salam, M. A. T. Masud, A. S. M. H. Masum, and M. M. Hossain. 2002. Performance of Some Grafted Eggplant Genotypes on Wild *Solanum* Root Stocks Against Root-Knot Nematode. *Journal of Biological Sciences* 2, no. 7 (): 446–448. ISSN: 17273048. doi:10.3923/jbs.2002.446.448. http://www.scialert.net/abstract/?doi=jbs.2002.446.448.

Renner, S. S., G. Chomicki, and W. Greuter. 2014. (2313) Proposal to conserve the name *Momordica lanata* (*Citrullus lanatus*) (watermelon, *Cucurbitaceae*), with a conserved type, against *Citrullus battich*. *Taxon* 63, no. 4 (): 941–942. ISSN: 0040-0262. doi:10.12705/634.29. http://doi.wiley.com/10.12705/634.29.

Renner, S. S., O. A. Pérez-Escobar, M. V. Silber, M. Nesbitt, M. Preick, M. Hofreiter, et al. 2019. A 3500-year-old leaf from a Pharaonic tomb reveals that New Kingdom Egyptians were cultivating domesticated watermelon. *bioRxiv*: 642785. doi:10.1101/642785. https://www.biorxiv.org/content/10.1101/642785v1.

Roberts, P. A. 2009. Concepts and consequences of resistance. In *Plant resistance to parasitic nematodes*, ed. by J. Starr, R. Cook, and J. Bridge. Wallingford: CABI. doi:10.1079/9780851994666.0023.

Robinson, R. W., and D. Decker-Walters. 1997. *Cucurbits*. 226. Wallingord, Uxon, U.K.: CAB International. ISBN: 0851991335.

Ros-Ibáñez, C., L. Robertson, M. d. C. Martínez-Lluch, A. Cano-García, and A. Lacasa-Plasencia. 2014. Development of virulence to *Meloidogyne incognita* on resistant pepper rootstocks. *Spanish Journal of Agricultural Research*. ISSN: 1695971X. doi:10.5424/sjar/2014121-4340.

Rubatsky, V. 2001. Origin, distribution, and uses. In *Watermelons: characteristics, production, and marketing*, ed. by D. Maynard, 227. ASHS Press. ISBN: 0970754612. https://www.cabdirect.org/cabdirect/abstract/20023058252.

Sabatino, L., E. Palazzolo, and F. D'Anna. 2013. Grafting suitability of Sicilian eggplant ecotypes onto *Solanum torvum*: Fruit composition, production and phenology. *Journal of Food, Agriculture and Environment*. ISSN: 14590263.

Sabatino, Iapichino, Rotino, Palazzolo, Mennella, and D'Anna. 2019. *Solanum aethiopicum* gr. gilo and Its Interspecific Hybrid with *S. melongena* as Alternative Rootstocks for Eggplant: Effects on Vigor, Yield, and Fruit Physicochemical Properties of Cultivar 'Scarlatti'. *Agronomy*. doi:10.3390/agronomy9050223.

Sain, R. S., P. Joshi, and E. V. Divakara Sastry. 2002. Cytogenetic analysis of interspecific hybrids in genus *Citrullus* (*Cucurbitaceae*). *Euphytica* 128 (2): 205–210. ISSN: 00142336. doi:10.1023/A:1020800113252.

Schomaker, C. H., and T. H. Been. 2013. Plant growth and population dynamics. In *Plant nematology*, ed. by R. Perry and M. Moens, 275–301. Ed. 2. Wallingford: CABI. doi:10.1079/9781845930561.0275. http://www.cabi.org/cabebooks/ebook/20063106966.

Seinhorst, J. W. 1970. Dynamics of Populations of Plant Parasitic Nematodes. *Annual Review of Phytopathology* 8, no. 1 (): 131–156. ISSN: 0066-4286. doi:10.1146/annurev.py.08.090170.001023. http://www.annualreviews.org/doi/10.1146/annurev.py.08.090170.001023.

— . 1998. The common relation between population density and plant weight in pot and microplot experiments with various nematode plant combinations. *Fundamental and Applied Nematology* 21:459–468. ISSN: 1164-5571. http://www.documentation.ird.fr/hor/fdi:010017629.

Shetty, K. D., and D. Reddy. 1985. Resistance in *Solanum* species to root-knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology* 15 (2): 230–230.

Shukla, N., R. Yadav, P. Kaur, S. Rasmussen, S. Goel, M. Agarwal, et al. 2018. Transcriptome analysis of root-knot nematode (Meloidogyne incognita)-infected tomato (Solanum lycopersicum) roots reveals complex gene expression profiles and metabolic networks of both host and nematode during susceptible and resistance responses. *Molecular Plant Pathology* 19, no. 3 (): 615–633. ISSN: 14646722. doi:10.1111/mpp.12547. http://doi.wiley.com/10.1111/mpp.12547.

Singh, A. K. 1978. Cytogenetics of semi-arid plants. III. A natural interspecific hybrid of *Cucurbitaceae* (*Citrullus colocynthis* Schrad*C. *vulgaris* Schrad). *Cytologya* 43 (3/4): 569–574. ISSN: 0011-4545. doi:10.1508/cytologia.43.569. http://joi.jlc.jst.go.jp/JST.Journalarchive/cytologia1929/43.569?from=CrossRef.

Singh, P., and T. Gopalakrishnan. 1997. Grafting for wilt resistance and productivity in brinjal (*Solanum melongena* L.). *Horticultural Journal* 10 (2): 57–64.

Solmaz, I., N. Sari, Y. Aka-Kacar, and N. Y. Yalcin-Mendi. 2010. The genetic characterization of Turkish watermelon (*Citrullus lanatus*) accessions using RAPD markers. *Genetic Resources and Crop Evolution* 57, no. 5 (): 763–771. ISSN: 0925-9864. doi:10.1007/s10722-009-9515-2. http://link.springer.com/10.1007/s10722-009-9515-2.

Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano, and J. Valero. 2005. Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology* 111, no. 1 (): 29–38. ISSN: 0929-1873. doi:10.1007/s10658-004-1982-x. http://link.springer.com/10.1007/s10658-004-1982-x.

Starr, J., and C. F. Mercer. 2009. 14 Development of Resistant Varieties. In *Root-knot nematodes*, ed. by R. Perry, M. Moens, and J. Starr, 326. Wallingford: CABI.

Szamosi, C., I. Solmaz, N. Sari, and C. Bársony. 2009. Morphological characterization of Hungarian and Turkish watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) genetic resources. *Genetic Resources and Crop Evolution* 56, no. 8 (): 1091–1105. ISSN: 0925-9864. doi:10.1007/s10722-009-9432-4. http://link.springer.com/10.1007/s10722-009-9432-4.

Talavera, M., S. Verdejo-Lucas, C. Ornat, J. Torres, M. D. Vela, F. J. Macias, et al. 2009. Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses. *Crop Protection* 28, no. 8 (): 662–667. ISSN: 02612194. doi:10.1016/j.cropro.2009.03.015. https://linkinghub.elsevier.com/retrieve/pii/S0261219409000763.

Tetteh, A. Y., T. C. Wehner, and A. R. Davis. 2010. Identifying Resistance to Powdery Mildew Race 2W in the USDA-ARS Watermelon Germplasm Collection. *Crop Science* 50 (3): 933. ISSN: 1435-0653. doi:10.2135/cropsci2009.03.0135. https://www.crops.org/publications/cs/abstracts/50/3/933.

Thies, J. A. 2011. Virulence of *Meloidogyne incognita* to expression of *N* gene in pepper. *Journal of nematology* 43, no. 2 (): 90–4. ISSN: 2640-396X. http://www.ncbi.nlm.nih.gov/pubmed/22791917%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3380459.

Thies, J. A., J. J. Ariss, R. L. Hassell, S. Buckner, and A. Levi. 2015a. Accessions of *Citrullus lanatus* var. *citroides* Are Valuable Rootstocks for Grafted Watermelon in Fields Infested with Root-knot Nematodes. *HortScience* 50, no. 1 (): 4–8. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.4. https://journals.ashs.org/view/journals/hortsci/50/1/article-p4.xml.

Thies, J. A., J. J. Ariss, R. L. Hassell, and S. Olson. 2009. Grafting for Management of Root-Knot Nematodes in Watermelon. *HortScience* 44 (3): 576. https://www.ars.usda.gov/research/publications/publication/?seqNo115=224817.

Thies, J. A., J. J. Ariss, C. S. Kousik, and R. L. Hassell. 2008. Grafting–a tool for managing root-knot nematodes in watermelon. *Phytopathology* 98:S156. https://www.ars.usda.gov/research/publications/publication/?seqNo115=224817.

Thies, J. A., J. J. Ariss, C. S. Kousik, R. L. Hassell, and A. Levi. 2016. Resistance to Southern Root-knot Nematode (*Meloidogyne incognita*) in Wild Watermelon (*Citrullus lanatus* var. *citroides*). *Journal of Nematology* 48 (1): 14–19. ISSN: 0022-300X. doi:10.21307/jofnem-2017-004. https://www.exeley.com/journal%7B%5C_%7Dof%7B%5C_%7Dnematology/doi/10.21307/jofnem-2017-004.

Thies, J. A., S. Buckner, M. Horry, R. Hassell, and A. Levi. 2015b. Influence of *Citrullus lanatus* var. *citroides* Rootstocks and Their F1 Hybrids on Yield and Response to Root-knot Nematode, *Meloidogyne incognita*, in Grafted Watermelon. *HortScience* 50, no. 1 (): 9–12. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.9. https://journals.ashs.org/view/journals/hortsci/50/1/article-p9.xml.

Thies, J. A., A. Levi, J. J. Ariss, and R. L. Hassell. 2015c. RKVL-318, a Root-knot Nematode-resistant Watermelon Line as Rootstock for Grafted Watermelon. *HortScience* 50, no. 1 (): 141–142. ISSN: 0018-5345. doi:10.21273/HORTSCI.50.1.141. https://journals.ashs.org/view/journals/hortsci/50/1/article-p141.xml.

Thies, J. A., and R. L. Fery. 1998. Modified expression of the *N* gene for southern root-knot nematode resistance in pepper at high soil temperatures. *Journal of the American Society for Horticultural Science* 123 (6): 1012–1015.

Trudgill, D. 1995. Origins of root-knot nematodes (*Meloidogyne* spp., Nematoda) in relation to their cultural control. *Phytoparasitica* 23 (3): 191–194.

Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. *Hilgardia* 7 (10): 389–415.

Tzortzakakis, E. A., F. A. Bletsos, and A. D. Avgelis. 2006. Evaluation of *Solanum* rootstock accessions for control of root-knot nematodes and tobamoviruses. *Journal of Plant Diseases and Protection*. ISSN: 18613837. doi:10.1007/BF03356178.

Uehara, T., M. Sakurai, K. Oonaka, Y. Tateishi, T. Mizukubo, and K. Nakaho. 2017a. Reproduction of *Meloidogyne incognita* on eggplant rootstock cultivars and effect of eggplant rootstock cultivation on nematode population density. *Nematological Research (Japanese Journal of Nematology)*. ISSN: 0919-6765. doi:10.3725/jjn.46.87.

Uehara, T., Y. Tateishi, Y. Kadota, and H. Iwahori. 2017b. Differences in parasitism of *Meloidogyne incognita* and two genotypes of *M. arenaria* on *Solanum torvum* in Japan. *Journal of Phytopathology*. ISSN: 14390434. doi:10.1111/jph.12594.

US Environmental Protection Agency. 2012. Ozone Layer Protection. Visited on 09/04/2019. https://www.epa.gov/ozone-layer-protection/.

Vela, M. D., A. Giné, M. López-Gómez, F. J. Sorribas, C. Ornat, S. Verdejo-Lucas, et al. 2014. Thermal time requirements of root-knot nematodes on zucchini-squash and population dynamics with associated yield losses on spring and autumn cropping cycles. *European Journal of Plant Pathology* 140, no. 3 (): 481–490. ISSN: 0929-1873. doi:10.1007/s10658-014-0482-x. http://link.springer.com/10.1007/s10658-014-0482-x.

Verdejo-Lucas, S., L. Cortada, F. J. Sorribas, and C. Ornat. 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of mi resistance gene tomato rootstocks under field conditions. *Plant Pathology*. ISSN: 00320862. doi:10.1111/j.1365-3059.2009.02089.x.

Verdejo-Lucas, S., M. Talavera, and M. F. Andrés. 2012. Virulence response to the *Mi.1* gene of *Meloidogyne* populations from tomato in greenhouses. *Crop Protection*. ISSN: 02612194. doi:10.1016/j.cropro.2012.03.025.

Walters, S., T. Wehner, M. Daykin, and K. Barker. 2006. Penetration rates of root-knot nematodes into *Cucumis sativus* and *C. metuliferus* roots and subsequent histological changes. *Nematropica* 36 (2): 231–242.

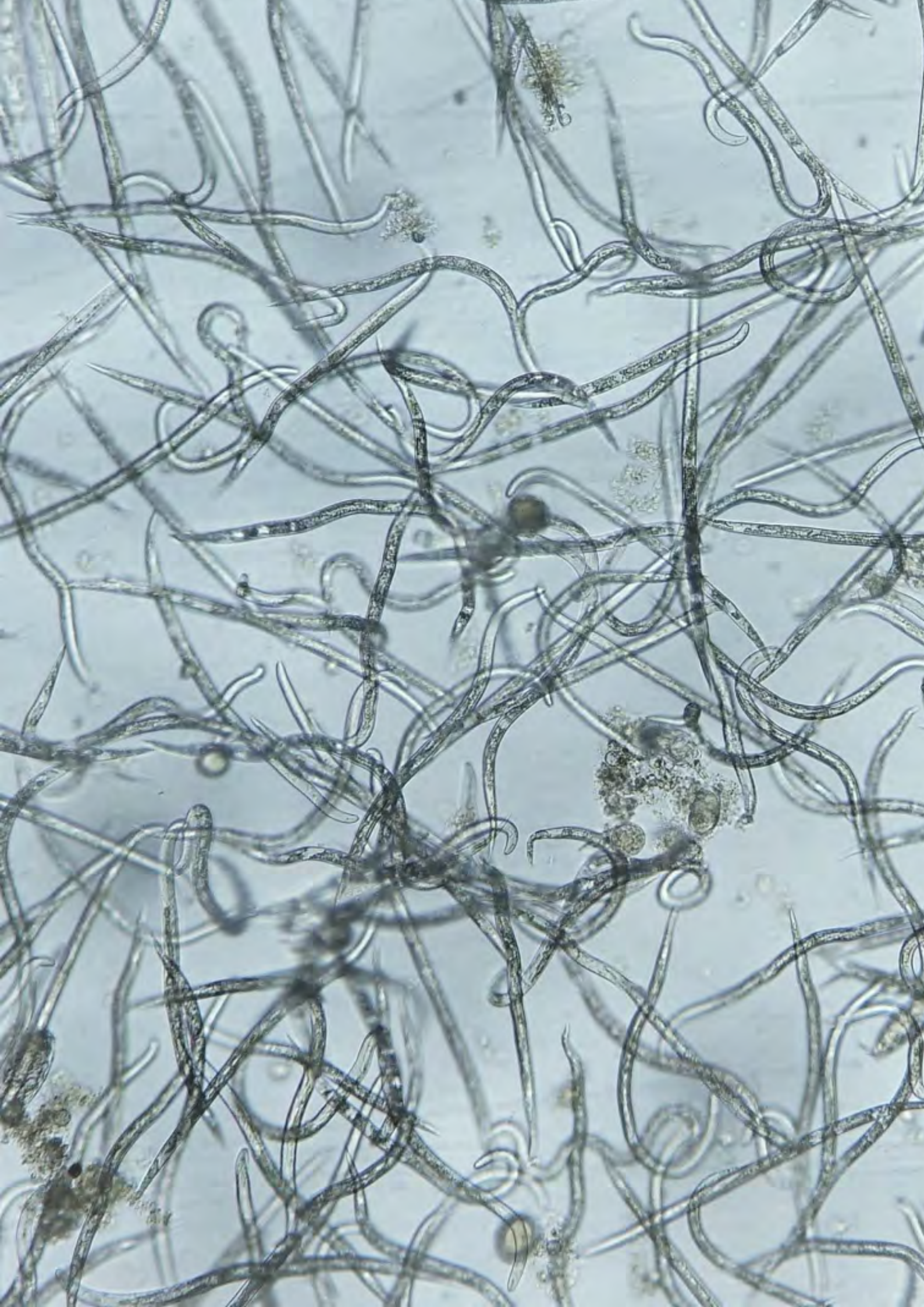
Wang, P., Q. Li, J. Hu, and Y. Su. 2015. Comparative analysis of genetic diversity among Chinese watermelon germplasmsusing SSR and SRAP markers, and implications for future genetic improvement. *Turkish Journal of Agriculture and Forestry* 39:322–331. ISSN: 1300011X. doi:10.3906/tar-1407-29. http://journals.tubitak.gov.tr/agriculture/issues/tar-15-39-2/tar-39-2-17-1407-29.pdf.

Weese, T. L., and L. Bohs. 2010. Eggplant origins: Out of Africa, into the Orient. *TAXON* 59, no. 1 (): 49–56. ISSN: 00400262. doi:10.1002/tax.591006. http://doi.wiley.com/10.1002/tax.591006.

Wehner, T. C. 2008. Overview of the genes of watermelon. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae*. https://w3.avignon.inra.fr/dspace/handle/2174/195.

Westphal, A. 2011. Sustainable approaches to the management of plant-parasitic nematodes and disease complexes. *Journal of Nematology* 43 (2): 122–125. ISSN: 0022-300X. https://journals.flvc.org/jon/article/view/78315.

Zhang, H., J. Fan, S. Guo, Y. Ren, G. Gong, J. Zhang, et al. 2016. Genetic Diversity, Population Structure, and Formation of a Core Collection of 1197 *Citrullus* Accessions. *HortScience* 51, no. 1 (): 23–29. ISSN: 0018-5345. doi:10.21273/HORTSCI.51.1.23. https://journals.ashs.org/view/journals/hortsci/51/1/article-p23.xml.



List of Tables

List of Figures