Suitability of Zucchini and Cucumber Genotypes to Populations of *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*

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Abstract: The host suitability of five zucchini and three cucumber genotypes to *Meloidogyne incognita* (MiPM26) and *M. javanica* (Mj05) was determined in pot experiments in a greenhouse. The number of egg masses (EM) did not differ among the genotypes of zucchini or cucumber, but the eggs/plant and reproduction factor (Rf) did slightly. *M. incognita* MiPM26 showed lower EM, eggs/plant, and Rf than *M. javanica* Mj05. Examination of the zucchini galls for nematode postinfection development revealed unsuitable conditions for *M. incognita* MiPM26 as only 22% of the females produced EM compared to 95% of the *M. javanica* females. As far as cucumber was concerned, 86% of the *M. incognita* and 99% of the *M. javanica* females produced EM, respectively. In a second type of experiments, several populations of *M. arenaria*, *M. incognita*, and *M. javanica* were tested on zucchini cv. Amalthee and cucumber cv. Dasher II to assess the parasitic variation among species and populations of *Meloidogyne*. A greater parasitic variation was observed in zucchini than cucumber. Zucchini responded as a poor host for *M. incognita* MiPM26, MiAL09, and MiAL48, but as a good host for MiAL10 and MiAL15. Intraspecific variation was not observed among the *M. javanica* or *M. arenaria* populations. Cucumber was a good host for all the tested populations. Overall, both cucurbits were suitable hosts for *Meloidogyne* but zucchini was a poorer host than the cucumber.
Key words: *Cucumis sativus*, *Cucurbita pepo*, infection, parasitic variation, reproduction, root-knot nematodes.

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

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

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

€640500 and €918300, respectively, in 2010 (Talavera et al., 2012).

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

MATERIALS AND METHODS

Host plants: Five genotypes of zucchini (*Cucurbita pepo* L.) and three of cucumber (*Cucumis sativus* L.) were selected for the study. The zucchini genotypes represented cultivars that produced fruits of different shapes and colors, and included cv. Amalthee (long light green), cv. Parador (long yellow), cv. Pixar (long green), cv. Floridor (round yellow), and cv. Satelite (round deep green). Cucumber genotypes represented cultivars that produced fruits of different length, cv. Taray (long), cv. Dasher II (medium long), and cv. Urano (short). Zucchini and cucumber seeds were soaked overnight and germinated in seed trays with vermiculite. When the first true leaf was fully expanded, the seedlings were transplanted to Styrofoam pots filled with 500 cm^3 of sterilized river sand. Plants were arranged randomly on a greenhouse bench and allowed to grow for 1 wk before nematode inoculation. They were watered daily as needed, and fertilized with a slow-release fertilizer (Osmocote® Scotts Company, Heerlen, Netherlands; 15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) at the beginning of the experiments.

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

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

Exp. 2 is a repetition of Exp. 1 with similar experimental conditions, plant maintenance and nematode assessments but was run for 74 d.

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

Parasitic variation in Meloidogyne: Thirteen RKN populations were tested on zucchini cv. Amalthee, and five on cucumber cv. Dasher II. These populations had been previously characterized for their Mi-gene virulence status (Ornat et al., 2001, Verdejo-Lucas et al., 2012), and were maintained as described previously. The identity of the populations was verified according to their enzymatic and molecular patterns (Esbenshade and Triantaphyllou, 1990; Zijlstra et al., 2000). They included the three most common species, *M. arenaria*, *M. incognita*, and *M. javanica*, in plastic greenhouses in southern Spain (Verdejo-Lucas et al., 2002; Talavera

et al., 2012). Plants were inoculated with 500 freshly hatched J2 (less than 72-hr old) and each treatment was replicated seven times. The experiment was conducted twice. Plants were harvested at 56 and 58 d after nematode inoculation. The root systems were washed free of soil, weighed, and stained in a 0.1-g/liter erioglaucine solution. The number of galls per root system with and without EM was counted. The infection frequency was calculated as described previously.

Statistical analyses: The SAS system V8 (SAS Institute Inc., Cary, NC) was used for statistical analyses. Analysis of variance was carried out by the general lineal model (Proc GLM). Normality was checked by the Shapiro-Wilk test and homogeneity of variances by the Levene's test. Data were transformed to (log x+1) when needed to improve homogeneity of error variances prior to the analyses. The experiments on host suitability were analyzed separately due to differences in egg hatching rate of the nematode inoculum. The response of the genotypes to each RKN isolate was compared and Tukey's honestly significant difference (HSD) test used to separate means ($P < 0.05$). Comparisons between RKN isolates on individual genotypes and between cucurbit crops for individual populations were done by Student's *t*-test ($P < 0.05$). The experiments on parasitic variation were analyzed together since no differences were found between the repeated experiments. The RKN populations were compared within

each cucurbit crop and means separated by Tukey's HSD test ($P < 0.05$).

RESULTS

Host suitability: Inoculated and noninoculated zucchini plants showed similar fresh top weight (data not shown), indicating that RKN infection and reproduction was not associated with plant damage after a single nematode generation. The hatching rate of the egg inoculum in Exp. 1 was 14% and 21% for *M. incognita* MiPM26 and *M. javanica* Mj05, respectively, which provided statistically different Pi values of 560 and 840 J2 per plant. The hatching rate in Exp. 2, 26.4% and 27.5% for *M. incognita* and *M. javanica*, respectively, provided similar Pi values of 1055 and 1099 J2 per plant, respectively. Nevertheless, the numbers of EM were similar statistically, although numerically different, among zucchini genotypes within RKN isolate and experiment (Table 1). On average, *M. incognita* showed similar infection frequency (%) in both experiments (8.6 ± 0.5 and 8.7 ± 0.5 in Exp. 1 and 2, respectively) despite differences in J2 inoculum as also did *M. javanica* (65 ± 3 and 72 ± 4 in Exp. 1 and 2, respectively). Zucchini genotypes differed in eggs/plant and Rf (Table 1), and the general trend in both experiments was that Amalthee supported less *M. incognita* eggs/plant than Satelite, whereas Parador, Pixar, and Floridor provided

TABLE 1.   Root weight, number of egg masses, eggs per plant and reproduction factor (Rf) of *Meloidogyne incognita* (MiPM26) and *Meloidogyne javanica* (Mj05) on genotypes of zucchini (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) in pot experiments (Exp.) conducted in a greenhouse for 65 and 74 d in Exp. 1 and 2, respectively.

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

Values are mean ± standard error of seven replicated plants/genotype. Values within each crop and experiment sharing the same letter are not significantly different according to Tukey's HSD test ($P < 0.05$).
[a] Eggs/plant divided by emerged second-stage juveniles.
* Statistical differences ($P < 0.05$) between *Meloidogyne* isolates.

intermediate values although statistical differences were only shown in Exp. 2. The Rf of *M. incognita* on Amalthee and Pixar was lower ($P < 0.05$) than on Satelite. The *M. javanica* eggs/plant were lower ($P < 0.05$) on Amalthee, Parador, and Pixar than on Floridor and Satelite in Exp. 2 (Table 1), and the Rf was lower ($P < 0.05$) on Amalthee than Parador, Pixar, or Floridor in Exp. 1, but only Amalthee differed from Floridor in Exp. 2 (Table 1). In relation to the RKN isolates, all reproductive traits of *M. incognita* MiPM26 were lower ($P < 0.05$) than those of *M. javanica* Mj05 in both experiments (Table 1). Female fecundity did not differ among the zucchini genotypes but *M. incognita* (253 ± 6 eggs/EM, mean ± standard error) showed lower ($P < 0.05$) fecundity than the *M. javanica* (538 ± 23 eggs/EM).

Inoculated and non-inoculated cucumber plants showed similar fresh top weight independently of the genotype. The hatching rate of the egg inoculum was 30.8% and 29.6%, and 26.4% and 27.5%, for *M. incognita* and *M. javanica,* in Exp. 1 and 2, respectively, which provided similar Pi values: 1234 and 1184 J2 per plant in Exp. 1, and 1055 and 1099 J2 per plant of *M. incognita* and *M. javanica,* respectively, in Exp. 2. The number of EM was similar among cucumber genotypes within RKN isolate in both experiments (Table 1). *M. incognita* produced less ($P < 0.05$) eggs/plant on Dasher II than Taray in Exp. 1, whereas *M. javanica* produced less eggs/plant on Dasher II than Urano in Exp. 2 (Table 1). The Rf of *M. incognita* on Dasher II was lower ($P < 0.05$) than that on Taray but only in Exp. 1 (Table 1). Cucumber genotypes did not differ in Rf values when infected by *M. javanica.* The fecundity of the females in the cucumber plants was similar among genotypes irrespective of the RKN isolate: 568 ± 48 and 553 ± 58 eggs/EM for *M. incognita* and *M. javanica,* respectively.

Postinfection development: Zucchini galls induced by *M. incognita* MiPM26 showed both small and large galls.

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

Parasitic variation in Meloidogyne: Zucchini showed a large variation in the number of galls (Table 3). As a general trend, the *M. javanica* populations showed the highest numbers of EM, followed by *M. incognita* MiAL10 and MiAL15, and those of *M. arenaria.* However, the exceptions to this trend were *M. incognita* MiPM26, MiAL09, and MiAL48 that showed high numbers of galls but few EM (Table 3). Cucumber showed a high correspondence between the numbers of galls and EM as more than 75% of the galls had EM. Only *M. arenaria* MaAL47 showed a lesser ($P < 0.05$) ability than the remaining populations to form galls and produce EM in the cucumber plants. When populations were grouped by *Meloidogyne* species (Table 4), *M. incognita* and *M. javanica* showed similar gall numbers on zucchini but higher ($P < 0.05$) than *M. arenaria.* The number of EM of *M. incognita* on zucchini was lower ($P < 0.05$) than that of *M. arenaria* followed by *M. javanica* (Table 4). On cucumber, *M. incognita* and *M. javanica* showed similar gall and egg mass numbers but *M. arenaria* produced less ($P < 0.05$) galls than *M. incognita,* and fewer ($P < 0.05$) EM

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

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

* Statistical differences between nematode species according to Student's *t*-test ($P < 0.05$).

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

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

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

than *M. incognita* and *M. javanica* (Table 4). The five individual populations tested on both cucurbit hosts showed lower (*P* < 0.05) EM numbers on zucchini than cucumber (Fig. 1), which is in support of the differential host status of these cucurbits to *Meloidogyne*. The average number of EM was 33 ± 3.4 on zucchini, and nearly thrice as many on cucumber (102 ± 12.8 EM).

DISCUSSION

The interaction between *Meloidogyne* spp. and two cucurbit crops was investigated in relation to plant genotype and RKN parasitic variation. Differences in host suitability among zucchini genotypes occurred consistently in nematode reproduction (eggs/plant and Rf) but not infection (EM and infection frequency) despite differences in ini-

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

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

Values are mean ± standard error of *n* × 7 replicated plants /experiment × 2 experiments. Values in the same column within each cucurbit crop followed by the same letter are not significantly different according to Tukey's HSD test (*P* < 0.05).

tial J2 inoculum levels. The lack of significant differences in Exp. 1 was probably due to the lower hatching rate of the egg inoculum in this experiment related to Exp. 2 which made any difference among genotypes more difficult to detect. In contrast, the RKN isolate had a striking effect on zucchini revealed by small infection frequency of 8.65% and Rf < 3.8-fold increase for *M. incognita* MiPM26 compared with a 65% to 72% infection frequency and Rf > 54-fold for *M. javanica* Mj05. The post-infection exam showed the inability of a high percentage of MiPM26 individuals to reach the egg-laying female stage on zucchini. Because 98% of the inoculated MiPM26 J2 penetrated the zucchini roots and developed into J4 within the first 11 days post-inoculation (López-Gómez and Verdejo-Lucas, 2014), we assumed that no mechanism was preventing root infection but the posterior development of the nematode once the feeding site was established. The slight genetic variability among these genotypes possibly reflects that fruit shape and size, phenotypic characters used for cucurbit domestication, have little effect on the host–nematode interaction.

The emptiness of a third of the galls could be due to crowding of large numbers of invading J2 leading to competition for available feeding sites, which may have stopped nematode development and eventually caused their death or affected their development into males (Stephan and Trudgill, 1982; Faske, 2013). Insufficient nutrient supply by non-fully functional feeding sites may have produced distorted females unable to lay eggs (McClure et al., 1974). Overall, the zucchini genotypes we tested provided unsuitable conditions for MiPM26
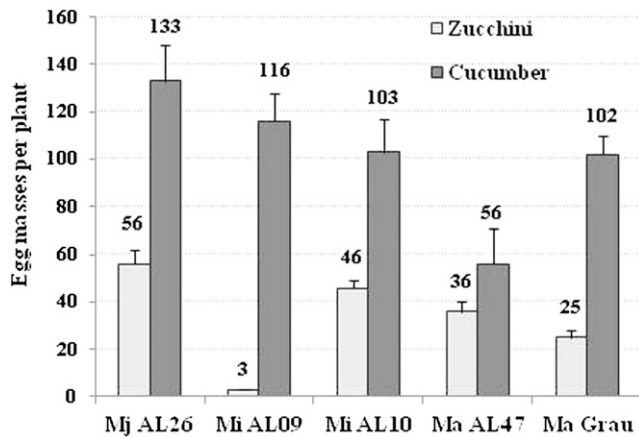
Fig. 1. Number of egg masses per plant, of *Meloidogyne* populations tested on both zucchini cv. Amalthee and cucumber cv. Dasher II inoculated with 500 second-stage juveniles in pot experiments conducted in a greenhouse. Bars represent the mean values plus standard error of 14 replicated plants per treatment. Cucurbit hosts differed in number of egg masses according to Student's *t*-test ($P < 0.05$).

development as did Amalthee for MiAL09 and MiAL48 infection. All zucchini genotypes, however, were excellent hosts for Mj05 and all *M. javanica* populations showed high infectivity on Amalthee. Strawberry genotypes that were susceptible to *M. hapla* were nonhosts to *M. incognita* (Edwards et al., 1985). Similarly, soybean genotypes were less suitable hosts to *M. incognita* than *M. arenaria* (Kirkpatrick and May, 1989).

The cucumber genotypes we tested showed similar susceptibility levels to both RKN isolates. However, cucumber cultivars grown in the Pothovar region of Pakistan differed in suitability levels to *M. incognita* with Rf from 0.33- to 10.52-fold increase in response to a 3,000 J2 inoculum (Mukhtar et al., 2013) which point out the genetic variability within this crop.

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

Root galling indicates successful establishment of the feeding sites that will allow further nematode development and life cycle completion. However, rating host suitability based on root galling may be misleading

(Fassuliotis and Dukes, 1972; Edwards et al., 1985; Fourie et al., 2012; Maleita et al., 2012), as gall formation is not always followed by successful nematode development. This was exemplified by *M. incognita* on zucchini that produced similar gall numbers but lower EM than *M. javanica*. Therefore, crop or cultivar recommendation cannot be made based only on the observation of root galling due to the RKN parasitic variation. In general, little host damage has been associated to low nematode reproduction (Ehwaeti et al., 1999), and differences among RKN populations have been observed on poor hosts or resistant genotypes such as pepper, asparagus, tomato, and celery (Khan and Khan, 1991; Dudash and Barker, 1992; Cortada et al., 2009; Melakeberhan and Wang, 2012).

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

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

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

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