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

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Abstract

The relationship between the initial (*P*_i) and final (*P*_f) population densities of *Meloidogyne javanica* in response to increasing initial inoculum levels and the effect on yield in zucchini cv. Amalthee (*Cucurbita pepo* L.) was determined using a geometric series of 12 *P*_i from 0 to 51,200 eggs/100 cm³ of soil in pot experiments in a greenhouse. The maximum multiplication rate was 425, and the equilibrium density was 701,951 eggs/100 cm³ soil. The relative yield, represented as dry top weight, fit the Seinhorst damage function model and the minimum relative yield (*m*) was 0.82 and the tolerance limit (*T*) was 402 J2/100 cm³ soil. Regression analyses indicated a negative relationship between the *P*_i and the leaf chlorophyll content (LCC) 40, 50, 60, and 70 days post-inoculation. The *P*_i and LCC fit the Seinhorst damage-function model. Zucchini cv. Dyamant was planted in a plastic greenhouse with a range of *M. javanica* *P*_i from 0 to 861 J2/100 cm³ soil. The maximum multiplication rate of *M. javanica* under field conditions was 3093, and the equilibrium density was 1485 J2/100 cm³ soil. The relationship between *P*_i and relative yield, represented as fruit weight, fit the Seinhorst damage function model (P < 0.0001, R² = 0.78); *m* was 0.48, and *T* was 0.02 J2/100 cm³ soil.

Keywords: *Cucurbita pepo*; Root-knot nematode; Damage functions

1 Introduction

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

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

As obligate sedentary endoparasites, RKN interfere with plant physiological processes involved in water uptake and nutrient translocation and create an imbalance of macro and micro-nutrients; in consequence, leaf chlorosis and stunted

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

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

2 Materials and methods

2.1 Pot experiments

A geometric series of 12 *Pi* of *M. javanica* was used to determine the relationship between *Pi* and *Pf* and yield in zucchini cv. Amalthee in a greenhouse. The experiment was conducted twice. Zucchini seeds were soaked overnight and germinated in seed trays with vermiculite. When the first true leaf was fully expanded, the seedlings were transplanted to Styrofoam pots filled with 500 cm³ of sterilized river sand. Plants were allowed to grow for one week before nematode inoculation. The *M. javanica* isolate (Mj05) had been established as a single egg mass and maintained on susceptible tomato cv. Roma in a greenhouse. The identification of the species was confirmed using SCAR-PCR markers (Zijlstra et al., 2000). To obtain the inoculum, eggs were extracted by blender maceration of infected roots using a 0.5% NaOCl solution for 5 min (Hussey and Barker, 1973). The egg suspension was passed through a 74 µm aperture sieve to remove root debris, and the dispersed eggs were collected on a 25 µm sieve. Seedlings were inoculated with 0, 50, 100, 200, 400, 800, 1600, 3200, 6400, 12,800, 25,600 and 51,200 eggs/100 cm³ soil. An additional level of 102,400 eggs/100 cm³ of soil was included in the replicated experiment. Each treatment was replicated seven times. Plants were maintained on a greenhouse bench, watered at field capacity before nematode inoculation, and after 5 days they were watered daily as needed, and fertilized with a slow-release fertilizer (Osmocote® Scotts Company, Netherlands; 15% N + 10% P₂O₅ + 12% K₂O + 2% MgO₂ + microelements) at the beginning of the experiments. Soil temperatures were recorded daily at 30-min intervals with temperature probes (Em50 Data Logger®, Decagon Devices Inc, USA, accuracy ± 1 °C, resolution 0.1 °C) inserted into the pots. The number of *M. javanica* generations was calculated according to Tb = 10.8 °C and 526 accumulated degree-days (DD) (Vela et al., 2014).

The hatching rate of the egg inoculum was determined by placing three aliquots of the egg suspension on Baermann trays that were maintained at 26 ± 1 °C in darkness for 21 days. Emerged second-stage juveniles (J2) were collected once a week and stored at 4 °C until counted. The hatching rate (%) was then calculated and the egg inoculum converted to number of emerged J2 that represented the effective inoculum for root invasion.

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

Plants were harvested 75 and 70 dpi in experiment 1 and 2, respectively. At harvest, tops were cut at ground level, oven-dried at 70 °C for 72 h and weighed. Roots were washed free of soil and the fresh weight recorded. Eggs were extracted from 10 g root subsamples using 0.5% NaOCl solution for 10 min as described previously and quantified (eggs and egg shells). The *Pi* is expressed as the number of emerged J2/100 cm³ soil and the *Pf* as the total number of eggs/100 cm³ soil.

2.2 Field experiments

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

Composite soil samples were collected at the beginning and end of the cropping cycle to estimate the *Pi* and *Pf* of *M. javanica*. Five soil cores were taken to 25 cm depth, mixed thoroughly and the nematodes extracted from 500 cm³ soil sub-samples in Whitehead trays (Whitehead and Hemming, 1965). One week later, the juveniles were collected, counted and expressed as J2/100 cm³ soil. At the end of the crop cycle, disease incidence was evaluated as the percentage of plants with galled roots, whereas disease severity was assessed using a root-gall index, according to a scale of 0 to 10, where 0 = a complete and healthy root system and 10 = dead plants and roots (Zeck, 1971). Roots from each plot were bulked, cut into 1 cm-long segments, and two 10 g subsamples were used to extract eggs by blender maceration in a 0.5% NaOCl solution for 10 min as described above. The number of eggs was expressed per gram fresh root weight.

Fruits were collected weekly for six weeks, weighed and the cumulated yield expressed as g/plant.

2.3 Statistical analyses

The SAS system V8 (SAS Institute Inc., Cary, NC) was used for statistical analyses. Data from pot experiments were presented separately due to differences between experiments in the hatching rate of the egg inoculum. Prior to the analyses, data were subjected to the normal Shapiro-Wilk W test to check for normal distribution of data and the ANOM for variances to check the homogeneity of variances. When needed, data were log transformed [log10 (x + 1)] to homogenize the variances. Data were subjected to ANOVA and means separated by the Tukey HSD (honest significant difference) test. Data from LCC were referred to that of uninoculated control plants to remove the effect of plant aging. The *Pi* values, ranging from 22 to 5687 J2/100 cm³ soil did not differ between experiments, and hence data within these *Pi* ranges were pooled and used to determine the relationship between *Pi* and *Pf*, and the fit of the data to the Seinhorst damage function model (Seinhorst, 1965). To estimate the maximum nematode reproduction rate (*a*) in zucchini, the *Pi* value with the highest slope in the regression line between *Pi* and *Pf* was selected, and the equilibrium density (*E*) was the value of *Pi* = *Pf* (Seinhorst, 1967). The maximum population density (*M*) was estimated from the experimental data, and *E*, according to the expression *M* = *aE*/(*a*–1) (Schomaker and Been, 2006). The nonlinear procedure (proc nlin) was used to fit data to the Seinhorst damage function model (Seinhorst, 1965); *y* = *m* + (1–*m*) *z* ^(*Pi*–*T*) when *Pi* ≥ *T*, and *y* = 1 when *Pi* < *T*, where *y* is the relative yield, *m* is the minimum relative yield, *z* is a constant ≤ 1, and *T* is the tolerance limit (the nematode density below which there is no yield loss). Similarly, nonlinear regression analyses were used to determine the relationship between *Pi* and LCC at each post-inoculation time, and if the relationship fitted the Seinhorst damage function model. The relative values of dry top weight, fruit weight and LCC were calculated for a given *Pi* at *Pi* = 0. Linear regression analysis was used to determine the relationship between gall rating and yield in the field experiment.

3 Results

3.1 Pot experiment

The minimum and maximum temperatures were 12 °C and 30.9 °C and 11.6 °C and 30.7 °C in experiment 1 and 2, respectively. The nematode had accumulated 738 and 734 DD when experiments 1 and 2 were harvested which indicated that *M. javanica* had completed one generation (526 DD) and started a second one. The hatching rate of the egg inoculum in the pot experiments was 11% and 43% which provided an effective J2 inoculum ranging from 6 to 5687 J2/100 cm³ soil and 22 to 44,032 J2/100 cm³ soil in experiments 1 and 2, respectively (Table 1) . *M. javanica* reproduced on zucchini cv. Amalthee at all *Pi* levels, and the maximum *Pf*/*Pi* of 532 (*Pi* = 11/100 cm³ soil) and 319 (*Pi* = 22 J2/100 cm³ soil) decreased to 97 (*Pi* = 5687/J2 100 cm³ soil) and 19 (*Pi* = 44,032 J2/100 cm³ soil) in experiments 1 and 2, respectively (Table 1). The maximum reproduction rate (*a*) and equilibrium density (*E*) of *M. javanica* was 425, and 701,951 eggs/100 cm³ soil, respectively (average of the two experiments, Fig. 1). Yield losses, represented as dry top weight, fit the Seinhorst damage function (P < 0.005, R² = 0.49), showing that *m* was 0.82 and *T* was 402 J2/100 cm³ soil (Fig. 2).

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

<i>Pi</i> (J2/100 cm³ soil) ^a	<i>Pf</i> × 10³/100 cm³ soil		<i>Pf</i> / <i>Pi</i>		Dry top weight (g)	
	1	2	1	2	1	2
0	0	0	0	0	5.5 ± 0.8 a	4 ± 0.5 ab
6	2 ± 4 e		428 a		5.1 ± 0.3 a	
11	6 ± 1.7 e		532 a		5.4 ± 0.4 a	
22	10 ± 2 e	7 ± 0.9 c	460 a	319 a	5.8 ± 0.4 a	3.9 ± 0.3 ab
45	16 ± 3 e	10 ± 1.7 c	366 ab	238 ab	6.1 ± 0.8 a	4.3 ± 0.3 ab
90	36 ± 6 e	19 ± 2 c	413 a	223 ab	5.5 ± 0.4 a	4.4 ± 0.3 a
181	67 ± 12 de	28 ± 1 c	379 ab	163 bc	5.3 ± 0.4 a	4.1 ± 0.3 ab
361	35 ± 6 e	61 ± 9 c	99 bc	177 bc	6.1 ± 0.9 a	4.3 ± 0.3 ab
723	266 ± 18 cd	112 ± 24 c	378 ab	162 bc	4.1 ± 0.3 ab	3.4 ± 0.2 ab
1446	359 ± 51 bc	184 ± 26 c	255 abc	134 bcd	5.1 ± 0.3 a	4.2 ± 0.2 ab
2893	755 ± 11 a	646 ± 42 ab	268 abc	235 ab	3.8 ± 0.3 b	3.3 ± 0.3 ab
5687	547 ± 97 ab	505 ± 54 b	97 bc	97 cde	4.3 ± 0.3 ab	3.5 ± 0.3 ab

11_008		562 ± 68 b		51 de		2.8 ± 0.4 ab
22_016		684 ± 107 ab		31 de		3.4 ± 0.4 ab
44_032		813 ± 11 a		19 e		2.5 ± 0.3 b

Values are mean ± standard error of seven replicates.

^a Egg inoculum converted to emerged juveniles according to a hatching rate of 11% and 43% in experiment 1 and 2, respectively.

Fig. 1 Relationship between initial (*Pi*) and final (*Pf*) population densities (J2/100 cm³ of soil) of *Meloidogyne javanica* in zucchini, *Cucurbita pepo*, cv. Amalthee inoculated with increasing inoculum levels of the nematode in pot experiments in a greenhouse. Values are means of 14 replicates/treatment.

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

The decline in LCC was associated with *Pi* and time (dpi) since the *Pi* × dpi interaction was significant ($P < 0.0001$). The analysis of the factor *Pi* on LCC indicated that *Pi* from 6 to 90 J2/100 cm³ soil had no significant effect on LCC in comparison to the uninoculated plants. Significant reductions of LCC were observed for *Pi* higher than 181 J2/100 cm³ soil (Table 2). As far as the effect of dpi was concerned, the LCC values progressively decreased over time with significant reductions between reading dates, except for 50 and 60 dpi (data not shown). The analysis of the *Pi* × dpi interaction showed significant declines at *Pi* ≥ 5687 J2/100 cm³ soil at 40 dpi, ≥ 1446 J2/100 cm³ soil at 50 and 60 dpi and ≥ 361 J2/100 cm³ soil at 70 dpi. The relationship between the *Pi* and LCC fit the Seinhorst damage function at each measured data (Fig. 3). A positive relationship between LCC and dry top weight was observed at 40 ($R^2 = 0.54$, $P > 0.0002$), 50 ($R^2 = 0.51$, $P < 0.0001$), 60 ($R^2 = 0.49$, $P < 0.0001$), and 70 dpi ($R^2 = 0.66$, $P < 0.0001$).

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

<i>Pi</i> (J2/100 cm ³ soil)	Days-post-inoculation			
	40	50	60	70
0	1 abc	1 ± 0.02 a	1 ± 0.04 a	1 ± 0.04 a
6*	1 ± 0.04 a	0.96 ± 0.03 a	1 ± 0.03 a	1 ± 0.05 a
11*	1 ± 0.02 a	0.96 ± 0.03 a	0.94 ± 0.04 ab	0.94 ± 0.06 abc
22	1 ± 0.05 ab	0.93 ± 0.03 ab	0.99 ± 0.05 ab	0.91 ± 0.04 abc
45	1 ± 0.04 ab	0.86 ± 0.03 a ^g e	0.92 ± 0.05 ab	0.96 ± 0.05 ab
90	1 ± 0.09 ab	0.87 ± 0.03 a ^g d	0.89 ± 0.05 ab	0.87 ± 0.04 abc
181	0.98 ± 0.03 abc	0.75 ± 0.03 c ^g g	0.83 ± 0.04 a ^g e	0.83 ± 0.05 a ^g d
361	0.92 ± 0.04 bcd	0.80 ± 0.02 b-f	0.86 ± 0.05 abc	0.73 ± 0.04 c ^g f
723	0.94 ± 0.04 a ^g d	0.80 ± 0.04 a ^g f	0.83 ± 0.03 a ^g d	0.64 ± 0.05 def
1446	0.90 ± 0.04 b ^g e	0.73 ± 0.05 d ^g h	0.74 ± 0.06 b ^g e	0.76 ± 0.05 b ^g e
2893	0.84 ± 0.03 c ^g f	0.58 ± 0.06 gh	0.60 ± 0.05 e	0.53 ± 0.05 efg
5687	0.81 ± 0.03 def	0.63 ± 0.03 fgh	0.62 ± 0.05 de	0.55 ± 0.04 fg
11,008*	0.71 ± 0.03 ef	0.61 ± 0.04 fgh	0.62 ± 0.06 cde	0.33 ± 0.04 gh
22,016*	0.65 ± 0.04 f	0.65 ± 0.08 e ^g h	0.66 ± 0.07 b ^g e	0.47 ± 0.08 fgh
44,032*	0.68 ± 0.03 f	0.52 ± 0.04 h	0.66 ± 0.04 b ^g e	0.24 ± 0.04 h

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

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

3.2 Field experiment

Pre-planting *M. javanica* population densities ranged from 0 to 861 J2/100 cm³ soil (477 ± 97 J2/100 cm³ soil, mean ± standard error), and the *Pf* from 0 to 7402 (2581 ± 944 J2/100 cm³ soil). The nematode was not detected in eight out of 24 plots at the beginning of the experiment and in three at the end of the cropping cycle. Eggs production ranged from 0 to 64,240 eggs/g root (18,630 ± 3585 eggs/g root). Disease incidence was 88% and disease severity was 4.9 with a range from 1 to 8. The relationship between

P_i and P_i/P_i was described by a potential function (Fig. 4), and the a and E values were 3093, and 1485 J2/100 cm³ soil, respectively. The relationship between P_i and the relative yield of zucchini cv. Dyamant, represented as fruit weight, fit the Seinhorst damage function model ($P < 0.0001$, $R^2 = 0.78$) showing that m was 0.48, T was 0.1 J2/100 cm³ soil, and the z value was 0.94 (Fig. 5). The relationship between root gall rating and yield was fitted to a linear function ($R^2 = 0.85$, $P = 0.0004$) with a negative slope where yield decreased with increasing gall rating (data not shown). Non-infected plants ($GI = 0$) produced an average yield of 3854 g/plant, similar to that of slightly infected plants ($GI < 4$), whereas plants heavily infected ($GI \geq 5$) yielded significantly less ($P < 0.001$) (Table 3). Yield of plants with gall ratings between 1–2 and 3–4 were 7% and 20% less than those of non-infected plants, respectively. The maximum yield losses were observed in plants with $GI \geq 7$.

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

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

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

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

Values of yield are mean ± standard error of n plants.

^a Based on a scale of 1 ~~to~~ 10 (Zeck, 1971).

4 Discussion

The population dynamic of *M.javanica* in zucchini was used to estimate host suitability by measuring the maximum reproduction rate and equilibrium density (Seinhorst, 1970); both parameters showed high values ($a = 425$ and $E = 701,951$ eggs/100 cm³ soil) in the pot experiments. However, they were not as high as those observed in good hosts such as cucumber ($a = 833$ and $E = 1,203,400$ eggs/100 cm³ soil) under plastic greenhouse conditions (Giné et al., 2014). In contrast, poor hosts such as watermelon showed comparatively low a and E values ($a = 14$ and $E = 49,400$ eggs/100 cm³ soil) (López-Gómez et al., 2014). The relationship between Pi levels and dry top weight resulted in a T value of 402 J2/100 cm³ soil, which suggests that zucchini is more tolerant to the nematode than other crops such as cantaloupe ($T = 19$ eggs and J2/100 cm³), tobacco ($T = 200$ eggs and J2/100 cm³) or cucumber ($T < 1$ J2/100 cm³) (Di Vito et al., 1983; Giné et al., 2014). Differences in m and T values observed between cv. Dyamant (0.48 and 0.02 J2/100 cm³ soil, respectively) and cv. Amalthee (0.82 and 402 J2/100 cm³ soil, respectively) probably reflect different experimental conditions, cultivar susceptibility or duration of the experiments (70 ~~to~~ 75 and 134 days in the pots and field, respectively), which suggests that yield losses would occur in zucchini if conditions for nematode development were suitable. The damage threshold in annual crops has been related to the planting date and the duration of the crop cycle (Ehwaeti et al., 1999; Talavera et al., 2009; Vela et al., 2014). The rotational crop preceding zucchini will also affect RKN reproduction and yield losses. For instance, squash produced less when preceded by RKN susceptible rather than resistant pepper (Thies et al., 2004). Similarly, cultivation of non-host crops before lemondrop squash reduced yield losses in squash (McSorley et al., 1994). Another consideration is the nematode survival rate during the short fallow periods between successive crops estimated in 50% of the Pf of the preceding crop (Ornat et al., 1999).

The negative relationship between gall rating and zucchini yield indicated that the root damage caused by RKN must be critical for the plant since it resulted in significant yield losses; 53% of the zucchini cv. Dyamant plants had $G \geq 5$ which indicated that 25% of the root system was not functional according to Zeck (1971). A similar relationship has been observed in other RKN susceptible crops such as carrots and peanuts (Bélair and Boivin, 1988; Korayem and Bondok Moawad, 2013). Although gall rating is not a quantitative measure, the correlation between root galling and zucchini yield supports the fact that rating root galling is a practical tool due to its relationship with yield losses. Root galling also provided detailed information on the spatial distribution of the nematode in the field and the identification of areas of severe disease symptoms that could allow the construction of a reference map to document the crop history of the site.

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

5 Conclusions

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

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References

Barker K.R. and Olthof T.H.A., Relationship between nematode population densities and crop responses, *Annu. Rev. Phytopathol.* **14**, 1976, 327–353.

Bélair G. and Boivin G., Spatial pattern and sequential sampling plan for *Meloidogyne hapla* in muck-grown carrots, *Phytopathology* **78**, 1988, 604–607.

Di Vito M., Greco N. and Carella A., The effect of population densities of *Meloidogyne incognita* on the yield of cantaloupe and tobacco, *Nematol. Medit.* **11**, 1983, 169–174.

Ehwaeti M.E., Fargette M., Phillips M.S. and Trudgill D.L., Host status differences and their relevance to damage by *Meloidogyne incognita*, *Nematology* **1**, 1999, 421–432.

Ferris H., Ball D., Beem L.W. and Gudmundson L.A., Using nematode count data in crop management decisions, *Calif. Agric.* **40**, 1986, 12–14.

Flor-Peregrin E., Azcón R., Martos V., Verdejo-Lucas S. and Talavera M., Effects of dual-inoculation of micorriza and endophytic, rhizospheric or parasitic bacteria on the root-knot nematode disease of tomato, *Biocontrol Sci. Technol.* **24**, 2014, 1122–1136.

Gholizadeh A., Amin M.S.M., Anuar A.R. and Aimrun W., Evaluation of leaf total nitrogen content for nitrogen management in a Malaysian paddy field by using soil plant analysis development chlorophyll meter, *Am. J. Agric. Biol. Sci.* **4**, 2009, 278–282.

Giné A., López-Gómez M., Vela M.D., Omat C., Talavera M., Verdejo-Lucas S. and Sorribas F.J., Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation, *Plant Pathol.* **63**, 2014, 1446–1453.

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

Kim D.G. and Ferris H., Relationship between crop losses and initial population densities of *Meloidogyne arenaria* in winter-grown oriental melon in Korea, *J. Nematol.* **34**, 2002, 43–49.

Korayem A.M. and Bondok Moawad M.M.M., Damage threshold of root-knot nematode, *Meloidogyne arenaria* on peanut in relation to date of planting and irrigation system, *Can. J. Plant Prot.* **1**, 2013, 117–124.

López-Gómez M. and Verdejo-Lucas S., Penetration and reproduction of root-knot nematodes on cucurbit species, *Eur. J. Plant Pathol.* **138**, 2014, 863–871.

López-Gómez M., Giné A., Vela M.D., Omat C., Sorribas F.J., Talavera M. and Verdejo-Lucas S., Damage functions and thermal requirements of *Meloidogyne javanica* and *M. incognita* on watermelon, *Ann. Appl. Biol.* **165**, 2014, 466–473.

Loveys B.R. and Bird A.F., The influence of nematodes on photosynthesis in tomato plants, *Physiol. Plant Pathol.* **3**, 1973, 525–529.

MAGRAMA, Estadística Agraria, *Superficies y producciones de cultivos* 2011, Ministerio de Medio Ambiente, Medio Rural y Marino <http://www.magrama.gob.es/es/estadisticas/estadisticas-agrarias/>, (accessed 30.03.14.).

McSorley R., Dickson D.W., De Brito J.A. and Hochmuth R.C., Tropical rotation crops influence nematode densities and vegetable yields, *J. Nematol.* **26**, 1994, 308–314.

Melakeberhan H., Physiological interactions between nematodes and their host plant, In: Chen Z.X., Chen S.Y. and Dickson D.W., (Eds.), *Nematology Advances and Perspectives, Nematode Management and Utilization* **vol. 2**, 2003, CAB International; Wallingford, UK, 771–794.

Melakeberhan H., Webster J.M. and Brooke R.C., Response of *Phaseolus vulgaris* to a single generation of *Meloidogyne incognita*, *Nematologica* **31**, 1985, 190–202.

Omat C., Verdejo-Lucas S. and Sorribas F.J., Effect of the previous crop on population densities of *Meloidogyne javanica* and yield of cucumber, *Nematropica* **27**, 1997, 85–90.

Omat C., Verdejo-Lucas S., Sorribas F.J. and Tzortzakakis E.A., Effect of fallow and root destruction on survival of root-knot and root-lesion nematodes in intensive vegetable cropping systems, *Nematropica* **29**, 1999, 5–16.

Ploeg A.T. and Phillips M., Damage to melon (*Cucumis melo* L.) cv. Durango by *Meloidogyne incognita* in Southern California, *Nematology* **3**, 2001, 151–157.

Pôrto M.L., Puiatti M., Fontes P.C.R., Cecon P.C., Alves J.C. and Arruda J.A., Índice SPAD para o diagnóstico do estado de nitrogênio na cultura da abobrinha, *Hortic. Bras.* **29**, 2011, 311–315.

Rharrabti Y., Villegas D., García del Moral L.F., Aparicio N., Elhani S. and Royo C., Environmental and genetic determination of protein content and grain yield in durum wheat under Mediterranean conditions, *Plant Breed.* **120**, 2001, 381–388.

Schomaker C.H. and Been T.H., Plant growth and population dynamics, In: Perry R.N. and Moens M., (Eds.), *Plant Nematology*, 2006, CAB International; Wallingford, 275–295.

Seinhorst J.W., The relation between nematode density and damage to plants, *Nematologica* **11**, 1965, 137–154.

Seinhorst J.W., The relationships between population increase and population density in plant parasitic nematodes. II. Sedentary nematodes, *Nematologica* **13**, 1967, 157–171.

Seinhorst J.W., Dynamics of populations of plant parasitic nematodes, *Annu. Rev. Phytopathol.* **8**, 1970, 131–156.

Sikora R.A. and Fernandez E., Nematode parasites of vegetables, In: Luc M., Sikora R.A. and Bridge J., (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, 2005, CAB International; Wallingford UK, 319–392.

Sorribas F.J. and Verdejo-Lucas S., Survey of *Meloidogyne* spp. in tomato production fields of Baix Llobregat county, Spain, *J. Nematol.* **26**, 1994, 731–736.

Sorribas F.J., Omat C., Verdejo-Lucas S., Galeano M. and Valero J., Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes, *Eur. J. Plant Pathol.* **111**, 2005, 29–38.

Talavera M., Verdejo-Lucas S., Omat C., Torres J., Vela M.D., Macias F.J., Cortada L., Arias D.J., Valero J. and Sorribas F.J., Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses, *Crop Prot.* **28**, 2009, 662–667.

Talavera M., Sayadi S., Chiroso-Ríos M., Salmerón T., Flor-Peregrín E. and Verdejo-Lucas S., Perception of the impact of root knot nematode induced diseases in horticultural protected crops of southeastern Spain, *Nematology* **14**, 2012, 517–527.

Thies J.A., Davis R.F., Mueller J.D., Fery R.L., Langston D.B. and Miller G., Double-cropping cucumbers and squash after resistant bell pepper for root-knot nematode management, *Plant Dis.* **88**, 2004, 589–593.

Vela M.D., López-Gómez M., Giné A., Sorribas F.J., Omat C., Verdejo-Lucas S. and Talavera M., Thermal time requirements of root-knot nematodes on zucchini-squash and population dynamics with associated yield losses on spring and autumn cropping cycles, *Eur. J. Plant Pathol.* **140**, 2014, 181–490.

Wagner A., Michalek W. and Jamiolkowska A., Chlorophyll fluorescence measurements as indicators of fusariosis severity in tomato plants, *Agro. Res.* **4**, 2006, 461–464.

Wani A.H., Management of root-knot nematode, *Meloidogyne incognita*, on okra and mung by soil amendment with oil cakes and leaves of different plants, *Nematol. Medit.* **34**, 2006, 83–87.

Whitehead A.G. and Hemming J.R., A comparison of some quantitative methods of extracting small vermiform nematodes from soil, *Ann. Appl. Biol.* **55**, 1965, 25–38.

Zeck W.M., A rating scheme for field evaluation of root-knot nematode infestations, *Pflanzenschutz-Nachr. Bayer. Ag.* **24**, 1971, 141–144.

Zijlstra C., Donkers-Venne D.T.H.M. and Fargette M., Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays, *Nematology* **2**, 2000, 847–853.

Highlights

- Zucchini squash showed tolerance to *Meloidogyne javanica* in protected cultivation.
- The leaf chlorophyll content declined with increasing initial population densities.
- The leaf chlorophyll content can be a practical tool to assess nematode damage.
- Root damage caused by *M.~~eloidogyne~~ javanica* was directly related with yield losses.
- Zucchini yield was significantly reduced in heavily infected plants.

Queries and Answers

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