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HOST RESPONSE TO *Meloidogyne incognita* INFECTION OF SEMI-TEMPERATE CARROT, CELERY AND SWEET PEPPER CULTIVARS COMMONLY GROWN IN THE HIGHLANDS OF BENGUET PROVINCE, PHILIPPINES

Nordalyn B. Pedroche[1,2], Ma. Teodora Nadong Cabasan[3]*, Luciana M. Villanueva[1] and Dirk De Waele[4,5]

[1]Department of Plant Pathology, College of Agriculture, Benguet State University, La Trinidad, Benguet 2601, Philippines

[2]Higher Education Regional Research Center, Benguet State University, La Trinidad, Benguet 2601, Philippines

[3]Department of Biological Sciences, College of Science and Mathematics, University of Southern Mindanao, 9407 Kabacan, Cotabato, Philippines

[4]Laboratory of Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), Belgium

[5]Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, 2520 Potchefstroom, South Africa

*Corresponding author: mtncabasan@usm.edu.ph

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ABSTRACT

Although the Philippines is a tropical country, 80% of semi-temperate vegetables are produced in the highlands (Cordillera) in northern Luzon. Due to the increasing yield losses caused by root-knot nematodes (RKN; *Meloidogyne* spp.) to these crops, a greenhouse experiment was conducted to evaluate the host response (resistance and tolerance) to *Meloidogyne incognita* infection of semi-temperate carrot, celery, and sweet pepper cultivars commonly grown in the Cordillera. The cultivars were inoculated with 1,500 eggs of *M. incognita* and the plants harvested 8 and 16 weeks after inoculation (WAI). Host response was determined based on the resistance index (RI; combining severity of root galling and number of eggs masses), the percentage change in plant growth and yield between inoculated and uninoculated plants. During the early stage of infection, the carrot cultivars were classified as either highly resistant or immune at 8 WAI. However, post-infectional response at 16 WAI showed the cultivars Caroline, Chunhong and Lucky Kuroda were classified as intermediate while Argo Super Kuroda and Royal Chantenay as moderately susceptible, and New Kuroda, New Kurodagosun and Victoria as susceptible. The celery cultivars Tall Utah Supreme and Utah 52-70 R Imp showed some resistance at 8 WAI, however, both cultivars were classified as susceptible at 16 WAI. The sweet pepper cultivar California Wonder was classified as moderately susceptible while Smooth Cayene and Yolo Wonder were classified as susceptible and highly susceptible, respectively at 8 WAI. The production of semi-temperate vegetables in the Cordillera is highly valuable. Knowledge of their host response to nematodes will assist farmers to minimize yield losses.

Key words: nematode reproduction, resistance, root galling, tolerance, yield loss

INTRODUCTION

The cultivation of semi-temperate vegetables in the Philippines is restricted mainly to two provinces (Benguet Province and Mountain Province) in the highlands (Cordillera) in the northern part of Luzon at 500 to 1,000 m above sea level. Benguet Province produces about 200,000 metric tons (mt) of semi-temperate vegetables each year (PSA 2018), which is 10 times more than Mountain Province. In the Cordillera, farmer s commonly plant semi-temperate vegetables along mountain slopes and in valleys forming terraces of vegetables. Given this type of terrain, the farmers apply conventional agronomic practices on small farms of about 2,500 to 5,000 m^2. In Benguet Province, the most commonly cultivated semi-temperate vegetables are cabbage (*Brassica oleracea* var. *capitata*; 80,634 mt), carrot (*Daucus carota*; 54,387 mt) and Chinese mustard (*Brassica chinensis*; 40,418 mt) (Philippine Statistics Authority 2018). Other important crops cultivated in the highlands of Benguet Province include celery (*Apium graveolens*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), snap beans (*Phaseolus vulgaris*) and sweet (or bell) pepper (*Capsicum annuum*).

Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasitic root pathogens that can cause serious damage to agricultural crops around the world (Jones et al. 2013). The root-knot nematode species *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are of particular economic importance to vegetable production worldwide (Hallmann and Meressa 2018). In the Philippines, *Meloidogyne* spp. have been reported associated with a number of high value vegetable crops in both the lowlands (Pascual et al. 2017) and the highlands (Pedroche et al. 2012). The economically damaging potential of this group of plant-parasitic nematodes was established on numerous vegetables including cabbage, carrot, cauliflower (*Brassica oleracea*), celery, eggplant (*Solanum melongena*), okra (*Hibiscus esculentus*), sweet pepper and tomato (*Solanum lycopersicum*; Davide 1988).

Plant resistance is considered by many as the best option for nematode management because of its cost effectiveness, compatibility with other management practices and benefit to environment (Starr and Mercer 2009). The first step consists of the identification of one or multiple sources of resistance which can be achieved by the evaluation of the host response of candidate cultivars, breeding lines, among others. This evaluation can lead to the identification of cultivars which can be grown in nematode-infested fields whether as the main crop or as a rotation crop. In addition, natural sources of resistance can also be used in breeding programs.

Although just a few sources of gene resistance especially single, against root-knot nematodes in vegetables are known (Hallmann and Meressa 2018), resistance to *Meloidogyne* spp. was reported in several temperate or semi-temperate vegetables. In carrots, the cultivars Brasilia and population derived from a cross between the European cultivars Scarlet Fancy and Favourite were found to be resistant to *M. incognita* (Parsons et al. 2015). Inbred lines derived from the open-pollinated cultivar Brasilia were used to produce progenies resistant to both *M. javanica* and *M. incognita* (Boiteux et al. 2000; Simon et al. 2000; Vieira et al. 2003). Two bell pepper (*C. annuum*) cultivars, Carolina Wonder and Charleston Belle, with high levels of resistance to *M. incognita* were approved for release by the Agricultural Research Service of the US Department of Agriculture (Fery et al. 1998). Potential resistance was found in five out of 19 carrot cultivars evaluated (Wesemael and Moens 2008). In sweet pepper, several dominant *R*-genes (the *Me* genes and the *N* gene) with resistance to root-knot nematodes have been characterised. Three of these genes (*Me1*, *Me3* and *N*) are routinely used in breeding pepper cultivars resistant to *M. arenaria*, *M. incognita* and *M. javanica* such as Carolina Wonder (Fery et al. 1998; for a summary see Barbary et al. 2016). Some Huacle and Serrano pepper lines were resistant to *M. incognita* (Gómez-Rodríguez et al. 2017). The *M. incognita* resistance locus Me7 has been mapped in a pepper population from a cross between the susceptible parent ECW30R and the -resistant parent CM334 (Changkwian et al. 2019). In celery, sources or mechanisms of resistance were not yet described (Bruznican et al. 2020).

In the Philippines, screening of some vegetables for natural resistance to *Meloidogyne* spp. was carried out by nematologists at Los Baños during the late 1960's and 1970's (Valdez 1968; Dela Rosa and Davide 1969; Toledo and Davide 1969; Ducusin and Davide 1972; Castillo 1976). However, screening for resistance to *Meloidogyne* spp. was mostly limited to lowlands crops and did not include semi-temperate vegetables which are only grown in the highlands of northern part of Luzon.

The study sought to evaluate the host response (resistance and tolerance) of three semi-temperate vegetables, *viz.* carrot, celery and sweet pepper, commonly grown today in the highlands of Benguet Province, to *M. incognita* infection. During a survey of the plant-parasitic nematodes associated with semi-temperate vegetables in the highlands of Benguet Province (Pedroche et al. 2012), this root-knot nematode species was identified as one of the predominant plant-parasitic nematode species infecting these crops.

MATERIALS AND METHODS

Plant material. The most common cultivars of carrot, celery and sweet pepper in Benguet Province were selected for the host response experiments (Table 1). Some have a long history of commercial production in the Cordillera whereas others were recently introduced. The seeds of celery and sweet pepper were pre-germinated in seedling trays containing heat-sterilised soil and after germination transplanted into 8-cm-diameter pots containing a heat-sterilised mixture (2,500 g) of garden soil and sand (1:1). The carrot seeds were directly seeded to 50 cm long pots (8 cm diam.). Plants were fertilized with complete fertilizer (14-14-14) at the recommended rate specific for vegetables at two splits as basal fertilizer and at hilling-up. Urea (46-0-0) (90 N ha^{-1}) and solophos (170 P kg ha^{-1}) were applied at the recommended rate during hilling-up. The plants were maintained at an ambient temperature of 26°C in a greenhouse and watered as needed.

Table 1. Cultivars of carrot, celery and sweet pepper included in the host response experiments.

Plant species	Cultivar
Carrot	Argo Super Kuroda, Caroline,
	Chunhong, Lucky Kuroda, New Kuroda
	New Kurodagosun, Royal Chantenay, Victoria
Celery	Tall Utah Supreme, Utah 52-70 R Imp
Sweet pepper	California Wonder, Smooth Cayene, Yolo Wonder

***Meloidogyne incognita* population.** The *M. incognita* population used in the experiments was originally isolated from galled celery roots cv. 'Tall Utah' grown in a commercial field in La Trinidad in the highlands of Benguet Province. A single egg mass was taken from a single gall and inoculated on a susceptible 4-week-old tomato cv. Apollo seedling potted in a greenhouse. Adult females were collected from the galled roots and identified as *M. incognita* based on the shape of the perineal pattern after 12 weeks (Hunt and Handoo 2009). When the single tomato plant had matured, the egg masses in the roots were isolated and used immediately to inoculate new 4-week-old tomato cv. Apollo seedlings.

Preparation of nematode inoculum. Eight- to 12-week-old galled tomato roots were gently uprooted, washed under running tap water to remove adhering soil particles and cut into 1- to 2-cm-long segments. Eggs were freed from the egg masses by dissolving the gelatinous matrices in 1% hypochlorite (NaOCl). The eggs-hypochlorite suspension was then poured through a series of nested sieves with 150 µm, 74 µm and 25 µm apertures. The eggs retained on the 25-µm-aperture sieve were collected and suspended in distilled water. The number of nematode eggs in the suspension was determined by counting the eggs in 1-ml-aliquots using a stereomicroscope.

Nematode inoculation. Per experiment, nine seedlings of each of the either direct-seeded (carrot) or transplanted (celery, sweet pepper) cultivars were inoculated 2 to 3 weeks after transplanting with 1,500 eggs pipetted into four 5-cm-deep holes made in the rhizosphere soil of the plants. In addition, nine seedlings of each of the cultivars remained uninoculated and served as control plants.

Assessment of root galling and nematode reproduction. The root systems of the plants were gently uprooted, washed under running tap water to remove adhering soil particles and examined for the presence of galls and egg masses at 8 weeks (carrot, celery, sweet pepper) and 16 weeks after inoculation (carrot, celery; WAI). The number of root galls and egg masses were counted using a stereomicroscope. On the basis of these numbers, root gall and egg mass index were determined based on a 0 to 5 score (Taylor and Sasser 1978; Table 2). Root sub-samples of 1 g taken at random per plant were stained with acid fuchsin (Byrd et al. 1983). Staining was only used for assessing the number of the different nematode developmental stages inside the roots while egg masses were counted without staining using a stereomicroscope. Rhizosphere soil sub-samples of 200 ml were taken at random per pot and the nematodes extracted using a modified Baermann tray method (Marais et al. 2017) and counted using a stereomicroscope.

Table 2. Root gall and egg mass index with corresponding assessment of the marketability of the yield of carrot tap roots.

Index	Number of root galls	Number of egg masses	Marketability of yield of carrot tap roots
0	0	0	no stunting: marketable
1	1-2	1-2	visible on the secondary roots with light forking, no stunting: marketable
2	3-10	3-10	none coalesced, forking, no stunting: marketable
3	11-30	11-30	some coalesced, severe forking and moderate stunting: marketable
4	31-100	31-100	many coalesced, severe stunting: unmarketable
5	> 100	> 100	mostly coalesced, severe stunting: unmarketable

Plant growth, yield and nematode damage. At termination of the experiments, plant height, fresh shoot and root mass, and yield were determined. For carrot and celery, data were gathered at 8 and 16 WAI to assess the early and late response to nematode infection; for sweet pepper that matures early, data were gathered at 8 WAI. To measure plant height (in cm), measurements were taken from the base of the plant to the highest leaf tip. To measure the fresh shoot and root mass, and yield (in g), a sensitive balance scale was used. Roots were washed with running tap water to remove the adhering soil particles and blotted dry with paper towels before weighing. Nematode damage was expressed as the percentage change in plant height, fresh shoot and root mass of nematode-infected plants compared with uninfected plants. The edible tap roots of the carrot cultivars were assessed visually for qualitative damage (forking) and their marketability classified as marketable and non-marketable (Bélair 1992; Table 2).

Host response. The evaluation of the host response of the plants to infection by *M. incognita* was based on the Resistance Index (RI; Kouamé et al. 1998; Table 3) which combines severity of root galling and number of egg masses into one single value using the equation: $RI = \sqrt{(\text{gall score}^2 + \text{egg mass score}^2)}$. The assessment at 8 WAI determined the host response at an early stage of infection.

When nematodes successfully established in the roots and reproduce, the late response to infection was assessed at 16 WAI.

Table 3. Host responses to *Meloidogyne incognita* infection based on the resistance index (RI)*

	Host response	RI
IM	immune	0-0.9
HR	highly resistant	1.0-1.9
R	resistant	2.0-2.9
MR	moderately resistant	3.0-3.9
I	intermediate	4.0-4.9
MS	moderately susceptible	5.0-5.9
S	susceptible	6.0-6.9
HS	highly susceptible	RI ≥ 7

*Kouamé et al. 1997.

Statistical analysis. There were three replicates per cultivar with three plants per replicate. The experiment was conducted in two trials, with consistent results, the data of the second trial were presented. The plants were arranged randomly in the greenhouse using a completely randomized design (CRD). The data were analysed statistically using Analysis of Variance (ANOVA) (STATISTICA Package Software). Differences among treatments were separated at $P \leq 0.05$ level of significance using Tukey's Honest Significant Difference (HSD) test. If the data had either no normal distribution or homogenous variance, even after log transformation and removal of the outliers, equivalent non-parametric analysis was performed. The Man-Whitney U test was used to compare two independent samples at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Carrot. Significant differences were observed in the mean number of galls per plant ($P \leq 0.05$) among the cultivars evaluated at 8 WAI (Table 4). New Kurodagosun had the highest number of galls (8) while no galls were observed on Royal Chantenay. The highest number of egg masses was also observed in New Kurodagosun. Significant ($P \leq 0.05$) differences were also observed in the mean number of J2 extracted from the rhizosphere soil among the cultivars evaluated. New Kuroda (72) and Victoria (60) had the highest number of J2 while New Kurodagosun (1) had the lowest number of J2. The number of swollen juveniles and adult females that had penetrated and developed inside the roots did not differ significantly among the cultivars evaluated. During the early stage of infection, at 8 WAI, after 2 generations of nematode life cycle, all carrot cultivars, except Argo Super Kuroda and New Kurodagosun, were classified as immune to *M. incognita* infection based on RI. Argo Super Kuroda and New Kurodagosun were classified as highly resistant. A significant ($P \leq 0.05$) increase in fresh shoot mass was observed in inoculated plants of New Kurodagosun (66.7%) and Victoria (52.1%) compared with uninoculated control plants (Table 5). Similarly, a significant ($P \leq 0.05$) marketable yield increase was observed in inoculated plants of New Kuroda (3.4%), Royal Chantenay (26.4%) and Victoria (33.3%).

Table 4. Average root galling, reproduction of *Meloidogyne incognita*, resistance index (RI) and host response (HoR) of eight carrot cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Swollen juveniles/g roots	Adult females/g roots	RI	HoR
Argo Super Kuroda	6 b	0^+ a	43 bc	0^+ a	6 a	1.2	HR
Caroline	2 ab	0 a	39 bc	0 a	1 a	0.4	IM
Chunhong	4 ab	0^+ a	15 b	1 a	3 a	0.7	IM
Lucky Kuroda	5 b	0^+ a	37 bc	1 a	9 a	0.9	IM
New Kuroda	3 ab	0^+ a	72 c	0 a	4 a	0.8	IM
New Kurodagosun	8 b	1 a	1 a	0^+ a	23 a	1.0	HR
Royal Chantenay	0^+ a	0 a	20 bc	0 a	4 a	0.1	IM
Victoria	2 ab	0 a	60 c	0 a	3 a	0.5	IM

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg mass score}^2)}$: IM = immune (RI = 0-0.9); HR = highly resistant (RI = 1-1.9) (Kouamé et al. 1997). $^+$ Value represents an average between 0 and 1. J2 = second-stage juveniles.

Table 5. Effect of *Meloidogyne incognita* on fresh shoot and primary root mass of eight carrot cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Shoot mass (g)			Primary root mass (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change
Argo Super Kuroda	11.2	12.8	(+)14.3 ns	18.3	16.9	(-) 7.7 ns
Caroline	10.1	12.0	(+)18.8 ns	22.0	18.9	(-)14.1 ns
Chunhong	10.1	11.8	(+)16.8 ns	20.4	21.7	(+) 6.4 ns
Lucky Kuroda	10.6	11.4	(+) 7.5 ns	16.8	14.2	(-) 15.5 ns
New Kuroda	15.0	14.0	(-) 6.7 ns	26.3	27.2	(+) 3.4 ns
New Kurodagosun	10.8	18.0	(+)66.7 *	24.6	29.7	(+)21.1 ns
Royal Chantenay	12.3	12.0	(-) 2.4 ns	24.7	31.2	(+)26.4 *
Victoria	7.1	10.8	(+)52.1 *	27.9	37.2	(+)33.3 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

At the late stage of infection, significant differences were observed in the mean number of galls and egg masses ($P \leq 0.05$) among the cultivars evaluated at 16 WAI (Table 6). The highest number of galls was observed in New Kuroda (693) which was significantly different ($P \leq 0.05$) from all other cultivars. The lowest number of galls was observed in Chunhong (43). The number of J2 extracted from the rhizosphere soil was significantly different ($P \leq 0.05$) among the cultivars evaluated. Royal Chantenay had the highest number of J2 (28) which was significantly different ($P \leq 0.05$) from all other cultivars evaluated except Argo Super Kuroda and Victoria. The lowest number of J2 extracted from the rhizosphere soil was observed in Caroline (1). The number of vermiform and swollen juveniles, and adult females that had penetrated and developed inside the roots was significantly different ($P \leq 0.05$) among the cultivars evaluated. The highest number of adult females was observed in New Kuroda (798) while the lowest number of adult females was observed in Lucky Kuroda (53). Based on RI, Caroline, Chunhong and Lucky Kuroda were classified as intermediately susceptible to *M. incognita* infection at 16 WAI. Argo Super Kuroda and Royal Chantenay were classified as moderately susceptible and New Kuroda, New Kurodagosun and Victoria as susceptible.

A significant increase ($P \leq 0.05$) in fresh shoot mass was observed in inoculated plants of Royal Chantenay (49.1%) compared with uninoculated control plants (Table 7). In seven out of the eight carrot cultivars tested, marketable yield was significantly reduced ($P \leq 0.05$) to 42.3% in inoculated plants compared with uninoculated control plants (100%).

Table 6. Average root galling, reproduction of *Meloidogyne incognita*, resistance index (RI) and host response (HoR) of eight carrot cultivars at 16 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Swollen juveniles/g roots	Adult females/ g roots	RI	HoR
Argo Super Kuroda	104 bc	65 ab	5 ab	41 bc	20 a	130 ab	5.8	MS
Caroline	113 bc	30 a	1 a	9 abc	154 c	226 ab	4.9	I
Chunhong	43 a	28 a	2 a	2 a	20 a	251 ab	4.5	I
Lucky Kuroda	63 ab	21 a	4 a	20 bc	21 ab	53 a	4.8	I
New Kuroda	693 d	157 b	5 a	16 bc	43 abc	798 c	6.5	S
New Kurodagosun	110 bc	52 ab	3 a	12 abc	60 bc	303 bc	6.2	S
Royal Chantenay	167 c	90 b	28 b	20 c	14 a	316 bc	5.8	MS
Victoria	174 c	65 ab	4 ab	5 ab	87 bc	214 bc	6.1	S

Means in the same column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg score}^2)}$: I = intermediate (RI = 4-4.9); MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9) (Kouamé et al. 1997). J2 = second-stage juveniles.

Significant differences were noted in the number of galls and egg masses among the eight cultivars evaluated. At the onset of disease development, host resistance can be shown in the suppression of nematode entry or nematode establishment in the feeding site. Host response in this study has been determined at 8 WAI to detect any resistance at the early stage of infection. At 8 WAI, only a few of the inoculated nematodes were able to establish feeding sites and formed galls in the roots but nevertheless, failed to reproduce and develop egg masses. At 16 WAI, after four generations of RKN have been produced, development and reproduction of RKN were observed as post-infectional response. The basis for determining the host as resistant is its ability to suppress nematode penetration, growth, development and reproduction. Based on RI, three of the cultivars were classified as intermediate, two as moderately susceptible and three as susceptible to *M. incognita* infection at 16 WAI. Thus, no source of resistance was found among the carrot cultivars evaluated.

Table 7. Effect of *Meloidogyne incognita* on fresh shoot mass and yield of eight carrot cultivars at 16 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Shoot mass (g)			Marketable yield (g)		
	-Mi	+ Mi	% change	-Mi	+Mi	% change
Argo Super Kuroda	19.3	15.8	(-) 18.1 ns	43.5	25.0	(-) 42.3 ns
Caroline	19.2	15.6	(-) 18.8 ns	58.9	35.8	(-) 39.2 ns
Chunhong	16.3	12.3	(-) 24.5 ns	38.2	21.3	(-) 44.2 *
Lucky Kuroda	17.8	16.8	(-) 5.6 ns	35.7	20.1	(-) 43.7 *
New Kuroda	19.0	19.3	(+) 1.6 ns	63.0	6.7	(-) 89.4 *
New Kurodagosun	30.0	23.0	(-) 23.3 ns	58.4	30.3	(-) 48.1 *
Royal Chantenay	10.8	16.1	(+)49.1 *	59.3	0	(-)100 *
Victoria	13.2	12.8	(-) 3.0 ns	62.3	13.9	(-) 77.7 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

Nematode infection promoted significantly higher primary root mass in carrot cultivars compared to uninoculated plants at 8 WAI. However, at the late stage of infection (16 WAI) differences in the marketable yield became evident. Infection by *M. incognita* significantly reduced the marketable yield of the carrot tap roots in six out of the eight cultivars evaluated at 16 WAI. The quality of the taproot is of major importance for the processing industry. Smooth, long, uncoalesced and unblemished tap roots are the quality requirements for commercial carrot production. Root-knot nematodes usually invade carrots at the root tips and induce (even at low numbers) secondary development resulting in forked and ramified tap roots, in addition to galls. Forking, twisting and cracking were also observed on *M. incognita*-susceptible carrots cultivars (Khan et al. 2018).

Formation of galls was evident around the lenticels of the primary roots with a somewhat light to dark brown discoloration in severe cases, in addition to forking. *M. chitwoodi* can cause severe galling near the lenticels resulting in a rough surface on the carrot taproot (Wesemael and Moens 2008). Lenticels are spongy regions in the periderm of stems and roots that allow gas exchange with the surrounding environment. The discoloration observed in our study may have been caused by secondary infections by fungi. The openings created by J2 of root-knot nematodes after penetration of the roots may serve as entry points for soil-borne microbial pathogens (Manzanilla-Lopez and Starr 2009). In addition to galling and forking of the tap roots, this discoloration further increases damage of these roots, thus decreasing the marketable yield and commercial value of root-knot nematode infected carrots.

Damage caused by *M. incognita* infection to carrots increases with increased duration of plant growth. A significant reduction in marketable root yield was observed in six out of the eight cultivars evaluated at 16 WAI *vs* only three cultivars at 8 WAI. This is consistent with an earlier study which reported that the damage in carrots caused by *M. chitwoodi* (inoculation density 25 J2/100 g soil) increased dramatically from 10 to 70% in a matter of 6 weeks (Wesemael and Moens 2008). Reducing the period that carrots are in the field by advancing the harvest date might significantly reduce the proportion of quality damage caused by *M. chitwoodi*. The life cycle duration of root-knot nematodes takes an average of 25 days at 27°C but may take longer at lower or higher temperatures (Ploeg and Maris 1999). Nematodes were given sufficient time to produce multiple generations creating more damage at 16 WAI (Wesemael and Moens 2008).

Celery. Only a significant difference ($P \leq 0.05$) in the number of J2 extracted from the rhizosphere soil was observed between the two cultivars evaluated at 8 WAI (Table 8). In Tall Utah Supreme, 60 J2/200 ml rhizosphere soil were counted *vs* 27 in Utah 52-70 R Imp. Based on RI, both cultivars were classified as resistant to *M. incognita* during the early stage of infection. No significant reductions in plant height, fresh shoot mass or yield were observed in inoculated plants compared with uninoculated control plants (Table 9).

Tall Utah Supreme had the highest number of galls ($P \leq 0.05$) (220 *vs* 143 in Utah 52-70 R Imp) and the highest number of J2 ($P \leq 0.05$) in the rhizosphere soil (180 *vs* 49 in Utah 52-70 R Imp) at 16 WAI (Table 8). However, the number of egg masses was significantly higher ($P \leq 0.05$) in Utah 52-70 R Imp (45 *vs* 19 in Tall Utah Supreme). Based on RI, Tall Utah Supreme was classified as moderately susceptible to *M. incognita* infection while Utah 52-70 R Imp was classified as susceptible during the late stage of infection. A significant reduction ($P \leq 0.05$) in fresh root mass (82.2%) and yield (72.9%) was observed in inoculated plants of Tall Utah Supreme compared with uninoculated control plants (Table 9).

Table 8. Average root galling and reproduction of *Meloidogyne incognita* on two celery cultivars 8 and 16 weeks after inoculation with 1,500 nematode eggs.

Celery cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Swollen juveniles per g roots	Adult females per g roots	RI	HoR
8 weeks								
Tall Utah Supreme	3 a	1 a	60 b	0 a	0 a	11 a	2.2	R
Utah 52-70 R Imp	4 a	1 a	27 a	0 a	2 a	15 a	2.2	R
16 weeks								
Tall Utah Supreme	220 c	19 b	49 ab	6.3 a	12 a	12 a	5.8	MS
Utah 52-70 R Imp	143 b	45 c	180 c	10 a	11 a	6 a	6.4	S

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg score}^2)}$: R = resistant (RI = 2-2.9); MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9) (Kouamé et al. 1997). J2 = second-stage juveniles.

Table 9. Effect of *Meloidogyne incognita* on plant height, yield, and fresh root mass of two celery cultivars 8 and 16 weeks after inoculation with 1,500 nematode eggs.

Celery cultivar	Plant height (cm)			Fresh root mass (g)			Yield (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change
8 weeks									
Tall Utah Supreme	37	39	(+)5.4 ns	24	30	(+)25.0 ns	111	124	(+)11.7 ns
Utah 52-70 R Imp	39	38	(-)2.6 ns	27	34	(+)25.9 ns	136	156	(+)14.7 ns
16 weeks									
Tall Utah Supreme	37	38	(+)2.7 ns	169	30	(-)82.2 *	250	68	(-)72.9 *
Utah 52-70 R Imp	31	31	0 ns	129	106	(-)17.8 ns	241	129	(-)46.5 ns

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

Based on RI, both celery cultivars evaluated showed resistance to *M. incognita* at the early stage of infection at 8 WAI. These celery cultivars however became susceptible on extended period of inoculation at 16 WAI. Under the prevailing environmental conditions in Benguet Province, it takes 16 weeks for the celery plants to mature. The significant increase in the number of galls, egg masses, vermiform and swollen juveniles in the roots between 8 and 16 WAI resulted in a significant reduction in fresh root mass and yield of one of the two cultivars evaluated. This observation indicates that damage caused by *M. incognita* infection on celery will increase with increased duration of plant growth. The cultivar Utah 52-70 R Imp included in our study is an improved offspring of Utah 52-70 H which is highly susceptible and sensitive to *M. incognita* infection (Perez and Castillo 1973; Castillo 1976). In our study, in spite of a significant increase in *M. incognita* infection at 16 WAI, plant height, fresh root mass and yield of Utah 52-70 R Imp was not significantly different compared with uninoculated control plants although the yield of Utah 52-70 R Imp reduced to 46% compared with uninoculated control plants. This observation may indicate that this improved celery cultivar has some level of tolerance to *M. incognita* infection but this should be further investigated. None of the infected roots of both cultivars evaluated showed necrosis. Instead, the production of secondary roots was more pronounced where galls were mostly observed.

Sweet pepper. At 8 WAI, nematode reproduction did not differ significantly among the three cultivars tested except the number of adult females in the roots (Table 10). The highest number of adult females in the roots was observed in Yolo Wonder (15) while the lowest number was observed in California Wonder (7). Based on RI, California Wonder was classified as moderately susceptible, Smooth Cayene as susceptible and Yolo Wonder as highly susceptible to *M. incognita* infection. Significant ($P \leq 0.05$) differences were observed among the three cultivars evaluated in plant height, fresh shoot and root mass, and yield between inoculated and uninoculated plants (Table 11). Plant height and fresh root mass were significantly reduced ($P \leq 0.05$) in Smooth Cayene, by 26.6 and 38.3%, respectively. Fresh shoot mass was significantly reduced ($P \leq 0.05$) in all three cultivars evaluated with 16.1 to 27.7%. In all three cultivars evaluated, yield in inoculated plants was reduced by almost 40% compared with uninoculated control plants but this reduction was only significant ($P \leq$ 0.05) in Yolo Wonder (39.7%).

Table 10. Average root galling and reproduction of *Meloidogyne incognita* on three sweet pepper cultivars at 8 weeks after inoculation with 1,500 nematode eggs.

Sweet pepper cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Adult females/ g roots	RI	HoR
California Wonder	85 a	33 a	16 a	0.1 a	7 a	5.7	MS
Smooth Cayene	124 a	91 a	33 a	0.3 a	10 ab	6.4	S
Yolo Wonder	211 a	123 a	34 a	0.4 a	15 b	7.1	HS

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = √(gall score2 + egg score2): MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9); HS = highly susceptible (RI ≥ 7) (Kouamé et al. 1997). J2 = second-stage juveniles

Based on RI, the sweet pepper cultivar California Wonder was classified as moderately susceptible to *M. incognita* infection while Smooth Cayene and Yolo Wonder were classified as susceptible and highly susceptible, respectively, at 8 WAI. *M. incognita* J2s were more attracted to sweet pepper cultivars, Yolo Wonder and California Wonder than other sweet pepper cultivars tested and both cultivars were highly susceptible to RKN exhibiting higher galling and egg mass indices (Kihika et al. 2017). Yolo Wonder had gall values similar to the susceptible reference sweet pepper accession and had the lowest marketable yield (Sánchez-Solana et al. 2016). In other reports (see for instance Barbary et al. 2014; Djian-Caporalino et al. 2001; Fazari et al. 2012) the host response of Yolo Wonder to *M. incognita* infection was described as partially resistant but this assessment was based on the low level of root galling induced upon infection and not on nematode reproduction (Barbary et al. 2016). In our study, both root galling and nematode reproduction on Yolo Wonder were very severe. Differences in host response of this cultivar could be attributed to the varying degree of virulence of *M. incognita* populations used in the experiments. In sweet pepper, the emergence of virulent populations of *M. incognita* breaking down the resistance induced by several dominant *R*-genes (*N* and *Me3*) has been reported (Castagnone-Sereno et al. 1996; Thies 2011).

Other nematode taxa were reported previously on semi-temperate vegetable crops in the Philippines. *M. javanica* and *M. hapla* were also abundant on carrot, celery, and sweet pepper (Pedroche et al. 2012). Host response of cultivars to other *Meloidogyne* species could vary from those observed on *M. incognita*. Host response at the time of harvest is important for consumption and marketing purposes.

Table 11. Effect of *Meloidogyne incognita* on plant height, fresh shoot and root mass, and yield of three sweet pepper cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Sweet pepper cultivar	Plant height (cm)			Shoot mass (g)			Root mass (g)			Yield (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change
California Wonder	62.7	56.3	(-)10.2 ns	56.4	40.8	(-)27.7 *	26.3	26.4	(+) 0.8 ns	115.6	70.1	(-)39.4 ns
Smooth Cayene	81.3	59.7	(-)26.6 *	52.2	39.8	(-)23.8 *	28.2	17.4	(-)38.3 *	45.8	28.0	(-)38.9 ns
Yolo Wonder	61.8	58.1	(-) 6.0 ns	52.2	43.8	(-)16.1 *	27.1	20.7	(-)23.6 ns	99.6	60.1	(-)39.7 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to Mann-Whitney U test. ns: not significant.

CONCLUSION

Our results demonstrate the huge impact *M. incognita* can have on carrot, celery, and sweet pepper which are highly-valuable cash crops for food security. Resistance in these crops to this root-knot nematode is scarce. However, where fields are heavily infested with *M. incognita*, a few varieties were identified (carrot cultivars Caroline, Chunhong and Lucky Kuroda; celery cultivars Tall Utah Supreme and Utah 52-70 R Imp of less than 16 weeks old; sweet pepper cultivar California Wonder) which are less susceptible to *M. incognita* infection. Field trials will further validate these promising cultivars which can be recommended to the farmers to help mitigate the damaging effects of this root-knot nematode on semi-temperate crops.

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