Host plant status and damage threshold of Pea (*Pisum sativum*) and Celeriac (*Apium graveolens* var. rapaceum) for the temperate root-knot nematode *Meloidogyne chitwoodi*

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Abstract

Outdoor vegetable cultivation is an important economic activity, both for fresh consumption and for the frozen vegetable industry within Flanders, Belgium. In recent years, due to the intensive nature of vegetable cultivation in the open air, nematode problems increased. The quarantine root-knot nematode *Meloidogyne chitwoodi* causes economic damage to the most important arable crops and vegetables grown in Flanders. However, for some vegetable crops knowledge on host plant status and damage potential is scarce or lacking. The host sensitivity for *M. chitwoodi* and damage thresholds of the most commonly field-grown cultivars of pea and celeriac were determined, both in glasshouse and field conditions. The damage threshold values (*T*) for the relative fresh pod weight of pea were 10, 1, 0.2 and 0 J2 (100 cm³ soil)$^{-1}$ for pea 'Cher', 'Electra', 'Geneva' and 'Pixia' respectively with up to 70% yield loss.. For celeriac 'Prinz', the damage threshold was 11.0 J2 (100 cm³ soil)$^{-1}$ with maximum 22% yield loss. Based on our pot trials, all pea cultivars were considered to be a good host with their high maximum population density (*M*) and maximum multiplication rate (*a*) values in the pot trials and reproductive factor (*Rf*) from the field trials. Celeriac proved to be an excellent host in the pot test but on the field the *M. chitwoodi* population declined. However, on plots with lowest yield a substantial number of *M. chitwoodi* was found in the roots. This is the first report of celeriac as host for *M. chitwoodi*.

Key words: Yield loss, host sensitivity, vegetables, plant-parasitic nematodes

Introduction

Plant-parasitic nematodes (PPN) are agricultural pests on a global scale and cause direct economic damage and crop loss for farmers. Over 4000 species of PPN have been recognized (Decraemer & Hunt, 2013). The global crop loss due to PPN was estimated from $US80 billion to $US157 billion annually (Nicol *et al*., 2011). Most PPN are very small, colorless and live in the ground which makes most farmers unaware of their presence in their fields and crops. On a global scale, root-knot nematodes (*Meloidogyne* spp.) are considered the most important PPN (Jones *et al*., 2013). *Meloidogyne* spp. have a damage potential of up to 100% at high densities, but serious damage can already occur at low densities. *Meloidogyne chitwoodi* was first discovered in the Pacific Northwest of the USA in 1980 (Golden *et al.*, 1980). Nowadays, *M. chitwoodi* is one of the most important pests and an EPPO A2 quarantine pest (Wesemael *et al.*, 2011). *Meloidogyne chitwoodi* has a wide host range and in Belgium it is concentrated in outdoor vegetable farming areas around Antwerp and Limburg but are also found in the provinces East- and West-Flanders (Waeyenberge & Moens, 2001). It is difficult to observe above ground symptoms in crops, though most crops can show disability in nutrient and water uptake because of galling on the root system induced by this nematode. The damage caused by *M. chitwoodi* is mainly quality damage that hinders the crop marketability (Wesemael *et al*., 2006). Wesemael and Moens (2008a) stated that under field conditions, at a relatively low density of 3 J2 of *M. chitwoodi* per 100 cm³ soil, 1.5% damage was already observed in carrots which corresponds to a financial loss of €500/ha. If a plot having carrots or black salsify is damaged more than 30%, the plot is no longer harvested which often leads to a financial loss of €2500-5500/ha. If potatoes are found to be infected the loss is around €4000/ha. Due to the wide host range, management is difficult. Many management strategies are being used to control *M. chitwoodi* and these include cultural management (den Nijs *et al.*, 2004), physical and biological control (Jaffee & Muldoon, 1995; Wishart *et al*., 2004), plant resistance (Janssen *et al*., 1995; Brown *et al*., 2006), chemical control (Griffin, 1989; Wishart *et al*., 2004) and prevention. Prevention includes avoidance of population build ups above damage thresholds. To deploy this strategy, knowledge on population dynamics and damage thresholds of *M. chitwoodi* for vegetable crops is needed.

Pea (*Pisum sativum*) is an important crop with a high level of protein that is used for both animal and human nutrition (Dahl *et al*., 2012). This annual vegetable can grow either alone or in intercropping systems. Peas are legumes that live in symbiosis with nitrogen fixing bacteria called Rhizobia to improve nitrogen availability in the soil. In northern Europe, seeds are sown in March and April to avoid frost damage. In southern Europe, they are sown in January and February. Normally peas are harvest with pea vining machines when the pods are filled with seeds and the seeds are fully expanded. Generally, farmers select crop variety based on region, sowing date, frost-, pest- and disease resistance or tolerance and market needs (Karkanis *et al*., 2016). Pea is a good host to *M. chitwoodi* (Santo & Ponti, 1985), *Pratylenchus penetrans* (Miller, 1978), *P. thornei*, *P. neglectus* and *P. hamatus* (Riga *et al*., 2008). The presence of these PPN often leads to yield loss.

Celeriac, *Apium graveolens* is among one of the plants with important pharmaceutical properties. The variety *rapaceum*, which is known as celeriac, is popular in Europe. It is an annual vegetable, best grown in cool weather and requires 90 to 120 days growing period before harvest. Due to this long growing period, they can be exposed to various soil borne pathogens. Not much information linked to damage by plant-parasitic nematodes nor the host status has been published on celeriac. Celery (*Apium graveolens*), which is a closely related crop, was reported to be infected by some root-knot nematodes such as *M. hapla*, *M. javanica* and *M. incognita* (Koshy *et al*., 2005; Vovlas *et al*., 2008).

Pea and celeriac are very important vegetables, both for the processing industry and for fresh market in Belgium, with an area of 11389 ha and 788 ha in 2021, respectively (Statbel, 2022). The aim of this research was to determine the host plant status of the most grown cultivars of pea and celeriac in Belgium for *M. chitwoodi* and determining the damage threshold for this nematode in both pot and field experiments.

Materials and methods

Culture of *M. chitwoodi* and inoculum preparation

The population of *M. chitwoodi* (Smakt) used in this research originated from a field in the Netherlands. They were maintained as a pure culture on tomato plants 'Marmande' under glasshouse conditions at 18 to 23°C with 16:8 hours of light and darkness, respectively, at the Institute for Agricultural and Fisheries Research (ILVO). The tomato plants were uprooted at least 3 to 4 months after the inoculation. The tomato roots were carefully washed and chopped into pieces (2 cm) and placed on Baermann funnels in a mist chamber to extract nematodes (Baermann, 1917). Only freshly hatched (<24 h) second-stage juveniles (J2) were collected and used as inoculum. The J2 density was determined by gently homogenising the nematode suspension gotten from the Baermann's funnels and counting three subsamples of 1 ml with the aid of a stereomicroscope. Inoculum densities were prepared following a log series 2^x (0, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 J2 per 100 cm^3 soil) for the pot experiments under glasshouse conditions.

Pot experiment on damage threshold and host plant status

Seeds from the pea cultivars and plants of celeriac used in these experiments were kindly provided by Belgian farmers. Four pea cultivars 'Cher', 'Electra', 'Geneva', 'Pixia' and one celeriac cultivar 'Prinz' commonly used in Flanders-Belgium were used in pot experiments to determine their host plant status and damage threshold for *M. chitwoodi*. A total of 220 pots (2 L and 15 cm in diameter) and 55 pots (5 L and 22.5 cm in diameter) were filled with 1500 cm^3 and 5400 cm^3 heat sterilised (100°C, 16 h) sandy loamy soil, respectively. Pea seeds (1 seed per pot with 55 pots per cultivar) were sown and celeriac plants (1 plant per pot with 55 pots used, plants at commercial planting size) planted. The experiments (celeriac and peas) took place in two separate glasshouses and the pots were completely randomised. The plants were watered upon requirements three times per week. The pots were all inoculated with freshly hatched J2 of *M. chitwoodi* following a log series 2^x (0, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 J2 per 100 cm^3 soil). Inoculation took place when true leaves had developed for peas. Celeriac plants were inoculated one day after the plants were transplanted in the 5 L pots. Three holes of 5 cm depth were made around the plants to easily apply the nematodes with the aid of a glass pipette. One week and five weeks after inoculation, 7g and 8g of a slow release fertilizer (N:P:K = 16:9:12) was given to pea and celeriac, respectively. For each nematode density five replicates per pea cultivar and celeriac cultivar were used. Pot experiments for pea took place in a glasshouse with controlled temperature conditions (day and night temperatures of 20-23°C and 16°C, respectively) and 12 h of light. Pot experiments for celeriac in the glasshouse were not subjected under controlled temperature conditions as pea. In both glasshouses the soil temperature was monitored with a data logger (Testo, Germany). At 8 and 31 weeks after sowing and planting, the pea pods and celeriac were ready to be harvested, respectively. The plants were carefully removed from the pots and both pea pods weight and celeriac tubers weight were measured. Soil was well homogenised and a subsample of 200 cm^3 was taken from each pot. The roots were carefully washed, weighed and cut in small pieces of about 2 cm and macerated at high speed for 60 seconds with a commercial Waring blender to liberate nematodes from the roots. With using an automated zonal centrifuge technique (Hendrickx, 1995), *M. chitwoodi* were extracted separately from soil subsamples and root samples. Nematodes extracted from both the organic and mineral samples were counted separately. To obtain the final density (*Pf*) in the pots, nematode counts from the soil subsample were extrapolated to the total soil volume and were added to the number of nematodes from the organic fraction. All stages of *M. chitwoodi* were counted.

Field experiment on damage threshold and host plant status

Two naturally infested fields with *M. chitwoodi*, located in the province of Limburg, Belgium, were used to evaluate the host plant status and damage threshold on two pea cultivars ('Cher' and 'Bartesa') and one celeriac cultivar ('Prinz'). In 2018, preliminary sampling was done to confirm that the only root-knot nematode species present was *M. chitwoodi*. Using the method of Wishart *et al.* (2002), the nematode species was molecularly confirmed. The fields were divided into 18 equal plots. The first field (field 1) had plots of 18 × 8 m and the second field (field 2) 12 × 8 m. In 2018, to obtain a low, intermediate and high *M. chitwoodi* density, *Tagetes patula*, maize and Italian ryegrass were sown, respectively. The initial population (*Pi*) per plot was determined before sowing pea or planting celeriac via soil sampling with the aid of a core (25 cm deep and 1.75 cm diameter). This was done following a zigzag sampling pattern by taking 60 soil cores from each plot. Peas were sown on different plots on the 1^{st}, 2^{nd} and 21^{st} of April 2019, Celeriac was planted on the 11^{th} of May 2019. Pea 'Cher' and 'Bartesa' reached their required industrial size in the field at 11 and 13 weeks after sowing, respectively, for celeriac 'Prinz' 26 weeks after planting were required. The yield of pea and celeriac per plot was determined by harvesting from 3 m^2 which was done 3 times per plot. Celeriac were also harvested twice from 1 m^2 per plot coupled with intensive soil sampling (60 soil cores $1m^{-2}$) to link the yield with nematode densities. From this 1 m^2, nematodes were extracted from both, roots (not tubers) and soil sample separately. The roots were cut from the tubers, weighed and chopped. Nematodes were than extracted from a subsample of 300 g after maceration at high speed during for 60 seconds with a commercial Waring blender to liberate nematodes from the roots. The final population for each plot (18 × 8m and 12 × 8m) from both fields was also determined by taking soil samples (60 soil cores/plot) to link the presence of *M. chitwoodi* with the yield in ton/hectare. Nematode extraction from both, mineral and organic samples, was done with the aid of an automated zonal centrifuge technique as described above. Only juveniles and males were counted. Eggs were not considered because it was not possible to differentiate *M. chitwoodi* eggs from eggs of other species based on egg morphology.

Data analysis and modelling

The data were analysed using R studio version 4.0.3. Data obtained for the damage threshold were fitted to the Seinhorst's model for yield equation (1) as described by Schomaker & Been (2013). Data for the host status were fitted to the equation (2) for sedentary nematodes with one generation per growing period of a crop (Seinhorst, 1967).

$$Y = Y_{max} \times (m + (1 - m) \times 0.95^{(Pi-T)/T}) \quad \text{for } Pi > T \qquad (1)$$

$$Y = 1 \qquad \text{for } Pi \leq T$$

$$Pf = M \times (1 - e^{-a \times Pi/M}) \qquad (2)$$

The weights of the pea pods and celeriac tubers were expressed as a fraction of the weight obtained for pea pods and celeriac tubers at $Pi = 0$ J2 per 100 cm^3 soil, described here as relative fresh pods or relative tuber weight. The pea pods and celeriac tubers weight were then averaged over replicates for each nematode density used. The maximum *Pi* density without noticeable damage (reduction of weight) was represented with the parameter *T* (= tolerance limit or damage threshold) with *m* for relative minimum yield and Y_{max} for relative maximum yield estimated using equation (1). The different nematode densities (*Pi*) with the weight of the plant parts (*Y*) are two variables in this equation (1). The relation between *Pi* (initial population) and *Pf* (final population) was modelled using equation (2) in order to estimate the maximum population density (*M*) and the maximum multiplication rate (*a*). The data were log transformed to obtain *Pf* from each nematode density averaged over all replicates and later back transformed.

The goodness of fit of the model is expressed by R^2 adjusted to degrees of freedom. Comparison among the parameter values was carried out at 5% level of uncertainty using LSD, the least square method.

Nematode reproductive factor (*Rf*) for pea and celeriac in field conditions were equally calculated by using the formula $Rf = Pf/Pi$. Based on the *Rf* values, the pea and celeriac cultivars were classified under five different categories (Schomaker & Been, 2013) as follows: Non-host = ($Rf < 0.15$), Poor host = ($Rf < 1.0 \geq 0.15$), Maintenance host = ($Rf \leq 2.0 \geq 1.0$), Good host ($Rf \leq 4.0 \geq 2.0$) and Excellent host ($Rf > 4.0$).

Results

Damage threshold and yield for pea and celeriac under glasshouse conditions

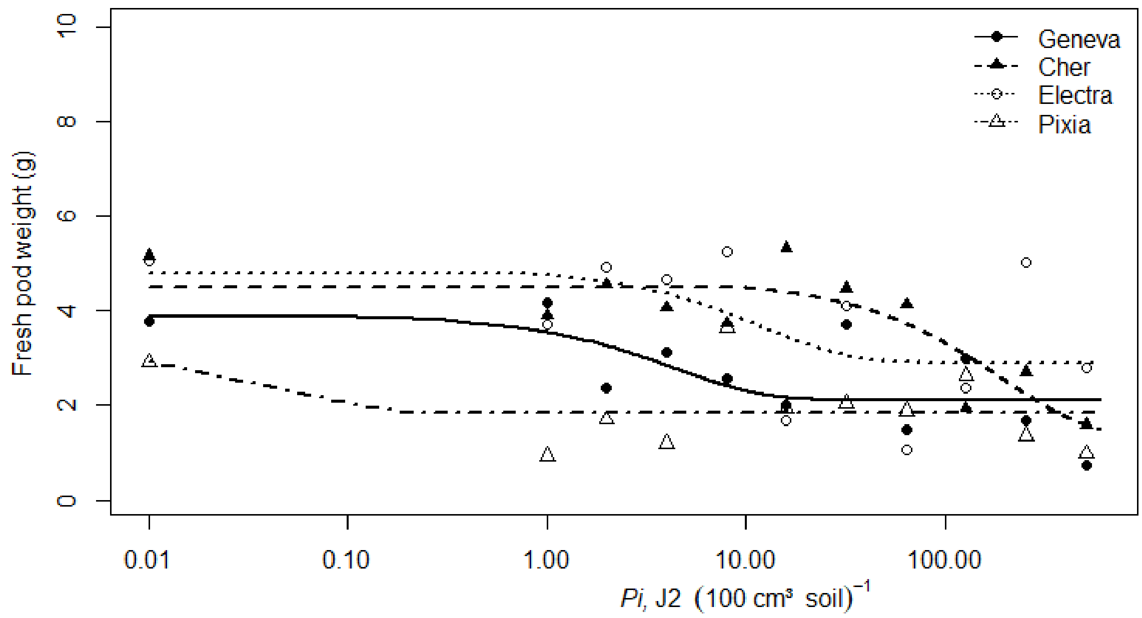
The effect of ranges of initial population densities (*Pi*) of *M. chitwoodi* on the fresh pea pod and celeriac fresh tuber weight was described by the Seinhorst model (equation 1) and is shown in Fig. 1a and 2a. The same data were relatively plotted (maximum weight at $Pi = 0$ is set as 1) for a better comparison between cultivars and estimation of potential yield loss as seen in Fig. 1b and Fig. 2b. All estimated parameters of the yield model with their standard errors (SE) and R^2 are summarised in Table 1. For pea 'Pixia' the model could not be properly fitted due to high variability.

The fresh pea pod weight per plant ranged between 0 and 9.04 g among the pea cultivars depending on the *Pi* (data not shown). The relative minimum pod weight (*m*) varied between 0.3 and 0.6, while the maximum yield (Y_{max}) ranged between 3.1 g and 4.8 g. The tolerance limit (*T*) varied from 0.2 and 10 J2 (100 cm^3 soil)$^{-1}$ for the pea cultivars. For celeriac 'Prinz' the fresh tuber weight ranged between 38.19 and 475.13 g at different *Pi* (data not shown). The relative minimum tuber weight (*m*) for celeriac 'Prinz' was 0.8 with a maximum yield (Y_{max}) of 226.16 g; the *T* was 11 J2 (100 cm^3 soil)$^{-1}$. There were no significant differences between the yield parameter values of pea cultivars, when 'Geneva', 'Electra' were compared with 'Cher' and 'Geneva' with 'Electra' as seen in Table 1. For pea 'Pixia' the standard error could not be calculated.

Table 1 Parameter values for the relation between *Pi* (juveniles) of *Meloidogyne chitwoodi* with pea fresh pod weight and celeriac fresh tuber weight obtained from Seinhorst's yield loss equation $Y = Y\text{max} \times (m + (1 - m) \times 0.95^{(Pi-T)/T})$: Where m = the minimum yield, T = tolerance limit in second-stage juveniles (cm³ soil)$^{-1}$ and Ymax = maximum yield (expressed in grams), including their standard error (SE), Non available (NA). The goodness of fit of the model is expressed by R^2 adjusted for degrees of freedom (df). Comparison among the parameter values only between the pea cultivars was carried out at 5% level of uncertainty using LSD least square method.

	m	T	Y_{max}	SE_m	SE_T	SE_{Ymax}	R^2	df	LSD_m	LSD_T	LSD_{Ymax}
Pea											
'Cher'	0.3	10	4.5	0.31	9.79	0.36	0.65	9	-	-	-
'Electra'	0.6	1	4.8	0.21	0.97	1.03	0.13	9	0.78|-	20.67|-	2.29|-
'Geneva'	0.5	0.2	3.8	0.18	0.41	1.03	0.22	9	0.75|0.25	20.58|2.08	2.29|3.09
'Pixia'	0.6	0	3.1	NA	NA	NA	-0.07	9	-	-	-
Celeriac											
'Prinz'	0.78	11.0	226.2	0.21	21.84	12.78	0.13	9	-	-	-

a



b

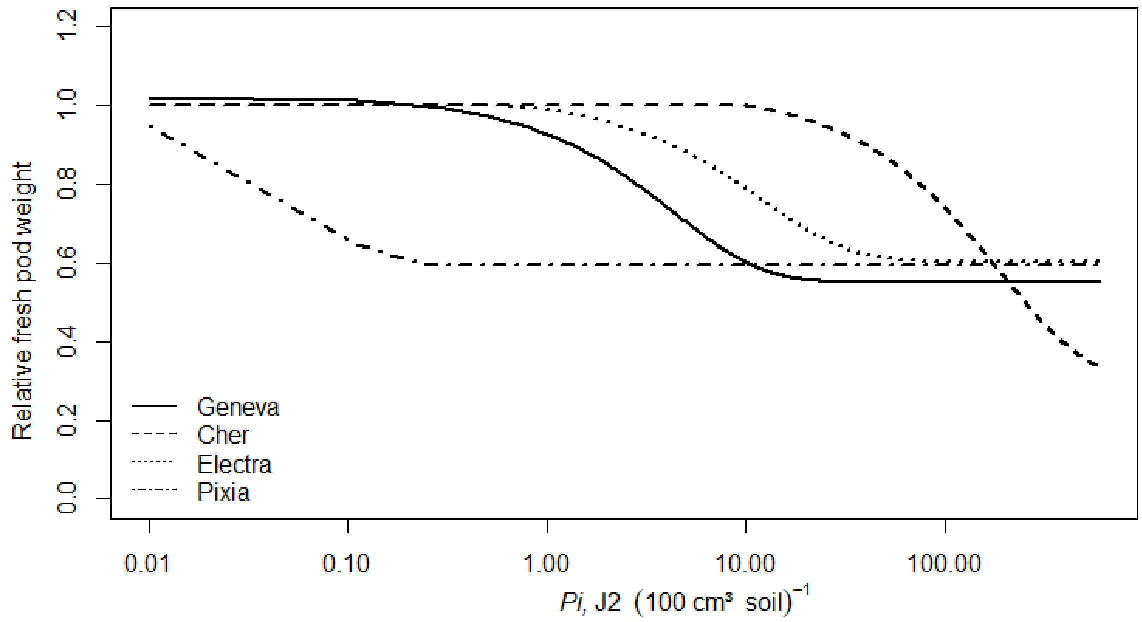
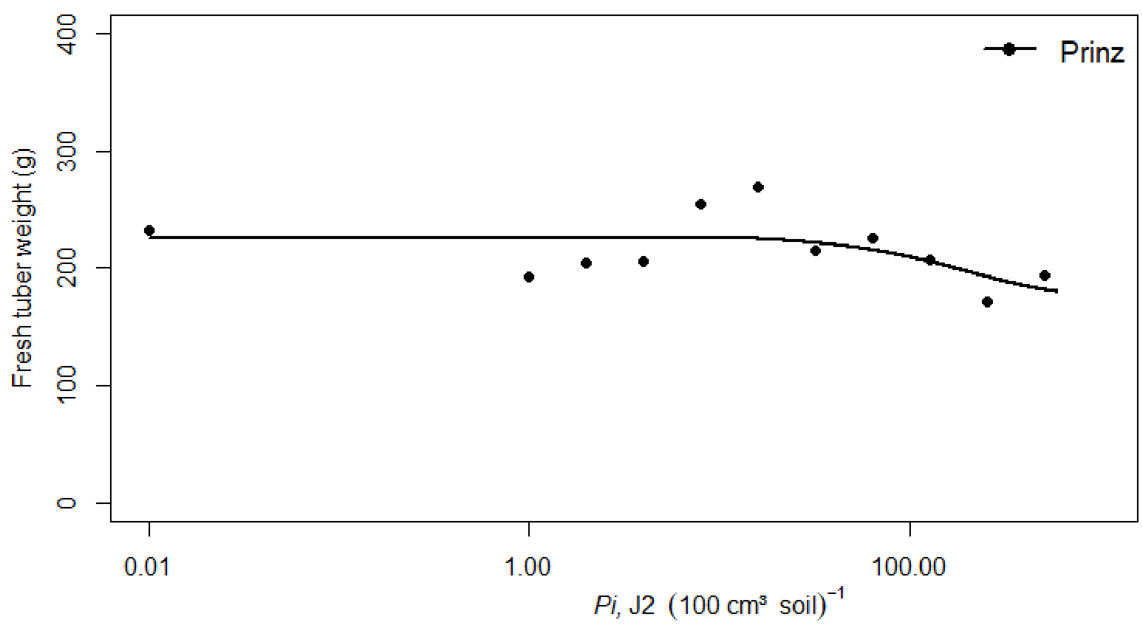


Fig. 1 The relationship between the *Meloidogyne chitwoodi* initial population density (*Pi*) on a log scale and yield with **a**: Fresh pod weight (g) and **b**: Relative fresh pod weight for four pea cultivars 'Geneva', 'Cher', 'Electra' and 'Pixia'. The fitted curve is according to Seinhorst equation. $Y = Y_{max} \times (m + (1 - m) \times 0.95^{(Pi-T)/T})$ for $Pi > T$ and $y = 1$ for $Y = 1$ for $Pi \leq T$.

a



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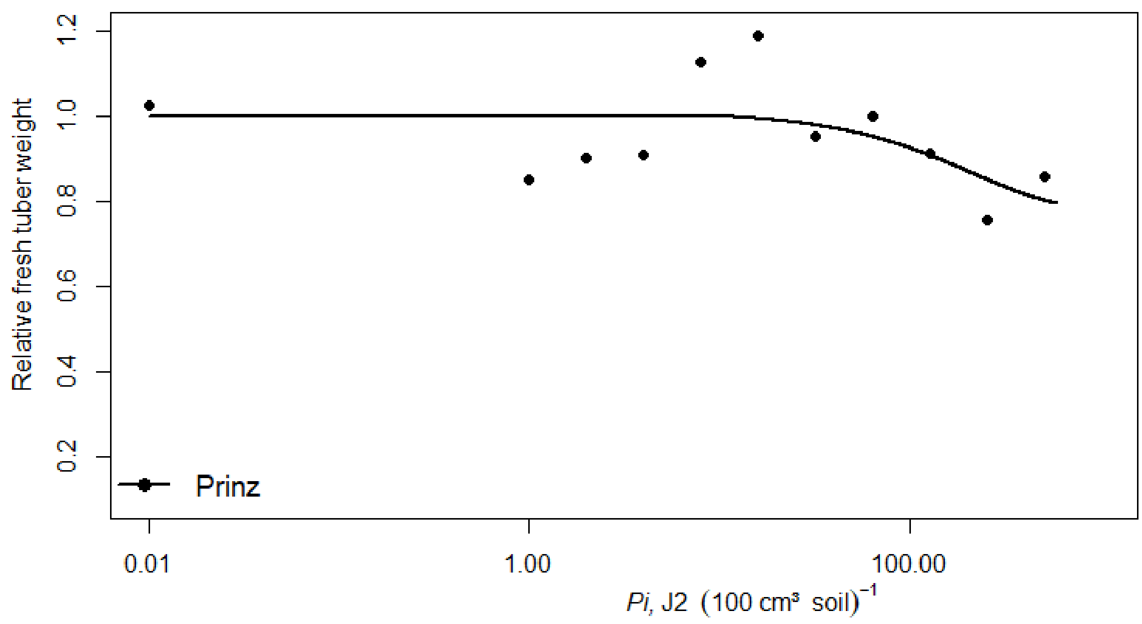


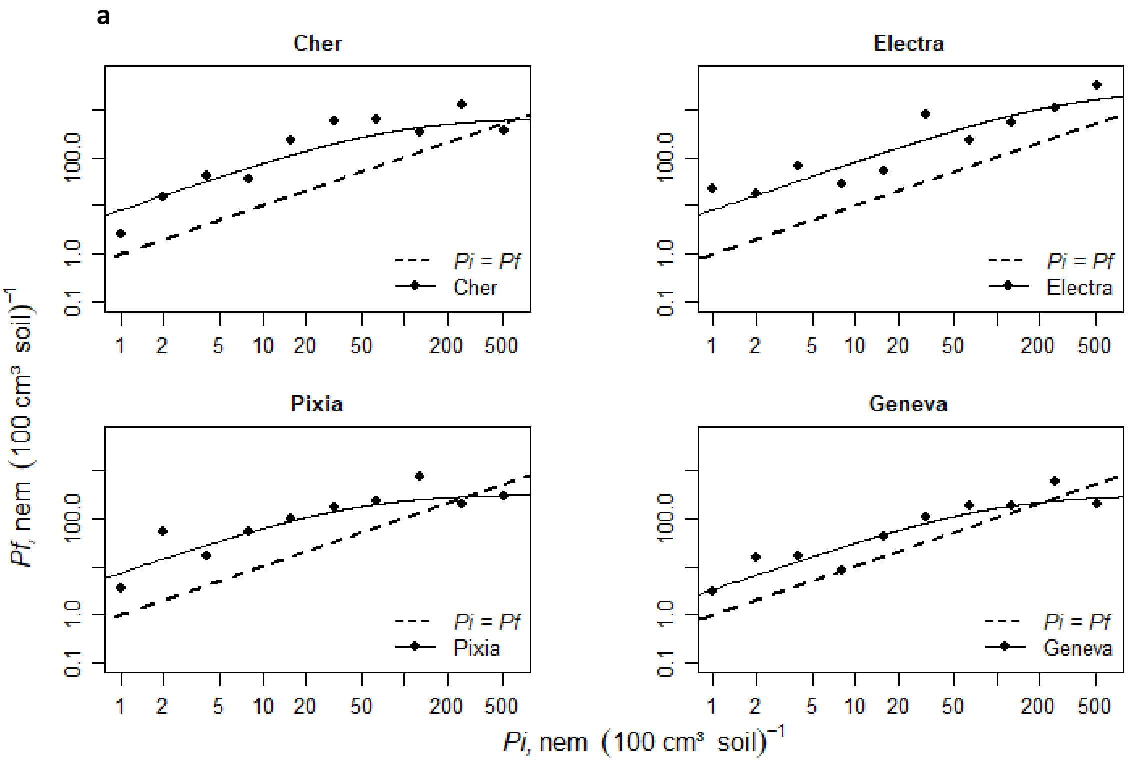
Fig. 2 The relationship between the *Meloidogyne chitwoodi* initial population density (*Pi*) on a log scale and yield with **a**: Fresh tuber weight (g) and **b**: Relative fresh tuber weight for celeriac 'Prinz'. The fitted curve is according to Seinhorst equation. $Y = Y_{max} \times (m + (1 - m) \times 0.95^{(Pi-T)/T})$ for $Pi > T$ *and y=* 1 for Y = 1 for $Pi \leq T$.

Population dynamics in pea and celeriac under glasshouse conditions

The relation between *Pi* and *Pf* for the four pea cultivars and celeriac 'Prinz' fitted well with the model for population dynamics (Equation 2) for sedentary nematodes with one generation with R^2 values ranging between 0.8 and 0.87 for pea cultivars and 0.63 for celeriac (Table 2). All the fitted curves for the four pea cultivars and one celeriac cultivar could be observed above the equilibrium density line ($Pf = Pi$) as shown in Fig. 3 and Fig. 4. The *Pf* ranged from 1 to 5555 *M. chitwoodi* (100 cm^3 soil)$^{-1}$ for the four pea cultivars and from 6 to 1463 *M. chitwoodi* (100 cm^3 soil)$^{-1}$ for celeriac 'Prinz' (data not shown). The maximum multiplication rate (*a*) ranged between 3.50 and 8.80 for the pea cultivars and it was 20.1 for celeriac. The maximum population density *M* for pea cultivars ranged from 402 to 3093 and that of celeriac was 124 *M. chitwoodi* (100 cm^3 soil)$^{-1}$ (Table 2). There were no significant differences established among the parameter values when all the pea cultivars were compared among them self which was carried out at 5% level of uncertainty using LSD least square method (Table 2).

Table 2 Parameter values of the population dynamics model for the relation between initial (*Pi*) and final population density (*Pf*) for four pea cultivars and one celeriac cultivar on a log scale obtained from Seinhorst's equation $Pf = M \times (1 - e^{-a \times Pi/M})$ for sedentary nematodes with one generation per growing period of a crop. Where *a* is the multiplication rate which is dimensionless while maximum population (*M*) is measured as *M. chitwoodi* (100 cm³ soil)$^{-1}$. Standard error (SE). The goodness of fit of the model is expressed by R^2 adjusted for degrees of freedom (df). Comparison of the parameter values at 5% level of uncertainty using LSD, the least square method was carried on comparing the pea cultivars 'Cher', 'Electra', 'Pixia' and 'Geneva' one on one.

	a	*M*	SE_a	SE_M	R^2	df	LSD_a	LSD_M
Pea								
'Cher'	8.49	994.95	2.78	598.95	0.85	8	-	-
'Electra'	8.8	3093.09	2.96	3555.72	0.81	8	8.61|-	7643.98|-
'Pixia'	8.04	401.67	2.68	179.32	0.8	8	8|8.46|-	1325.4|7547.37|-
'Geneva'	3.5	418.67	0.92	212.57	0.87	8	6|6.56|6.01	1347|7551.25|589.55
Celeriac								
'Prinz'	20.1	124.2	7.89	29.2	0.63	8	-	-



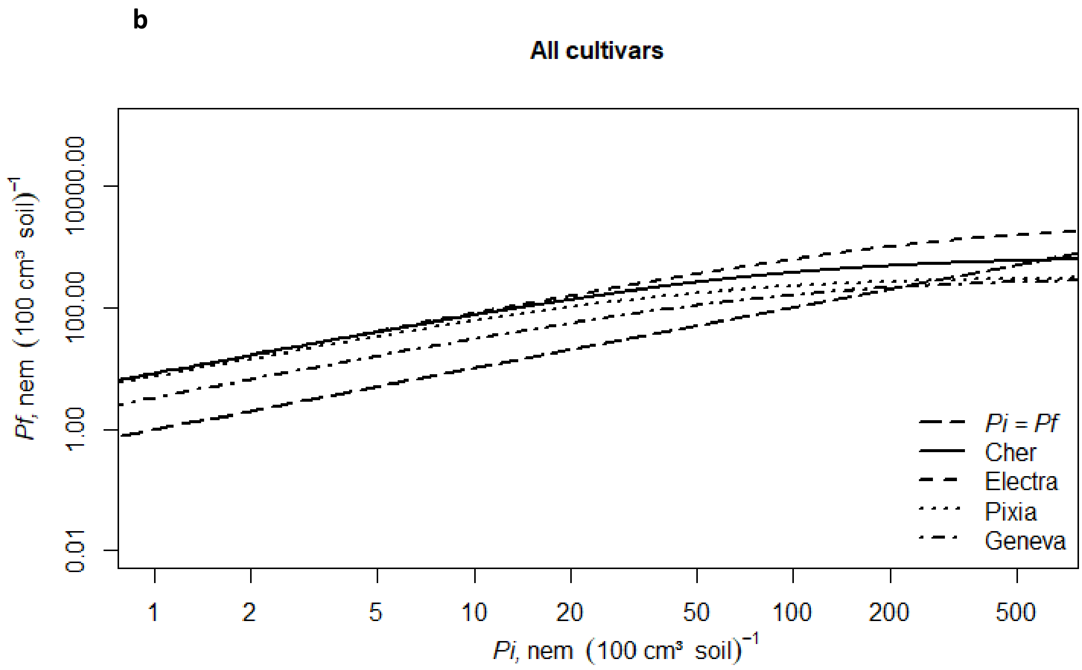


Fig. 3 Relationship between initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne chitwoodi* on a log scale: **a** = For the four pea cultivars 'Geneva', 'Cher', 'Electra' and 'Pixia' and **b** = All cultivars put together on one graph. Fitted curves according to the equation: $Pf = M \times (1 - e^{-a \times Pi/M})$ for population dynamics. The straight diagonal dashed line represents the population equilibrium line at $Pf = Pi$

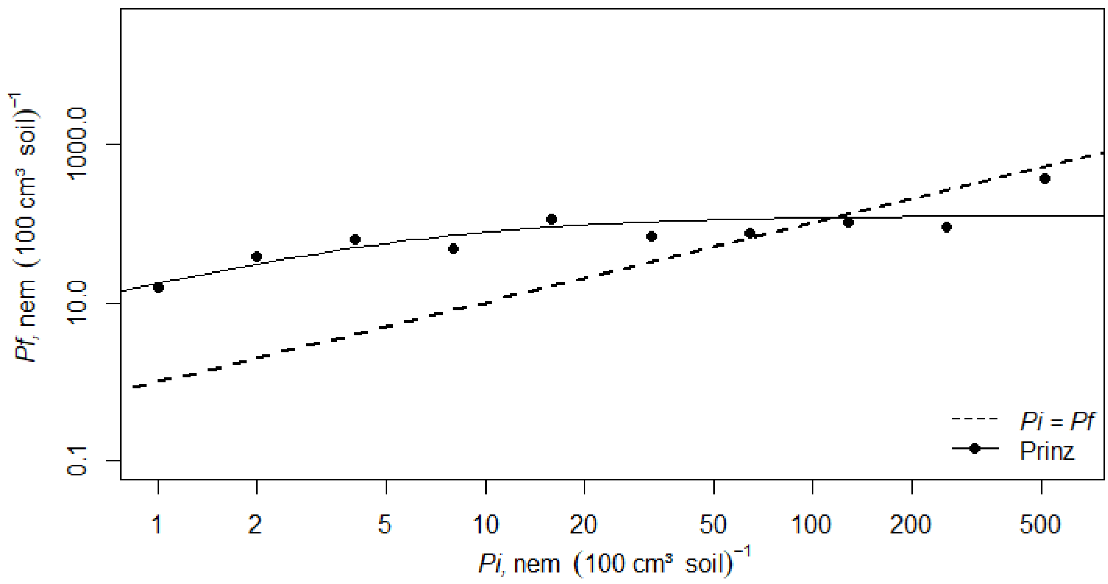


Fig. 4 Relationship between initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne chitwoodi* on a log scale for celeriac 'Prinz'. The curve fitted according to the equation: $Pf = M \times (1 - e^{-a \times Pi/M})$ for population dynamics and the straight diagonal dashed line represents the population equilibrium line at $Pf = Pi$

Damage threshold and host plant status under field conditions

For pea, we did not collect intensive samples from 1 m^2 as the peas were already wilted on the field. For celeriac 'Prinz' there was no correlation observed between the fresh tuber weight and the *M. chitwoodi* population (100 cm^3 soil)$^{-1}$ from each micro plot. A negative correlation was observed between the fresh tuber weight and the nematodes in the root system in field 1 (Table 3). In field 2 most nematodes per gram of root were also found in the plot with the lowest average yield but this was not pronounced. The yield on field 1 was higher than on field 2.

Table 3 The mean fresh tuber weight (g) (± standard deviation) and nematode counts (*Meloidogyne chitwoodi*) per 1 m^2 (± standard deviation) of celeriac 'Prinz' after harvest in roots and in 100 cm^3 soil from two different fields with plot sizes of 18 × 8 m (field 1) and 12 × 8 m (field 2).

Plot	Field 1			Field 2		
	***Pf* in roots (per g root)**	***Pf* in soil (per 100 cm³)**	**Yield (g)**	***Pf* in roots (per g root)**	***Pf* in soil (per 100 cm³)**	**Yield (g)**
1	0.7 ± 0.5	50 ± 17	840 ± 60	0 ± 0	595 ± 528	528 ± 138
2	0.3 ± 0.1	269 ± 87	1054 ± 116	5 ± 2	34 ± 20	510 ± 35
3	4.1 ± 3.7	43 ± 27	531 ± 308	5 ± 3	117 ± 53	274 ± 29

Marketable yield of peas and celeriac from the fields was determined from subsamples of 3 m^2 and is shown in Tables 4 and 5. For pea 'Cher' yield was not determined as plants were already wilted on the field. The field period was 93 days with 930 degree days with base temperature 5°C (DD_5) (field 1) and 98 days with 933 DD_5 (field 2) for pea 'Bartesa', and 74 days with 835 DD_5 for 'Cher' (field 1). For celeriac 'Prinz' the field period was 155 days with 2161 DD_5 on field 1 and 189 days with 2416 DD_5 on field 2. In general, the yield of peas and celeriac on field 1 was higher than on field 2. Celeriac on field 2 suffered from carrot fly (*Chamaepsila rosae*).

On field 1 there was a significant lower yield of pea 'Bartesa' on the plot with the highest *Pi*. Much lower yields were obtained in field 2, irrespective of *Pi*. The host plant status of both pea cultivars varied under field conditions from poor host to excellent host based on *Pi*/*Pf* values from J2. The calculated *Rf* values ranged from 0.29 to 7.76 for pea with an increase of the population on field 1 and a decrease on field 2.

Table 4 The mean initial (*Pi*) and final (*Pf*) population of *Meloidogyne chitwoodi* per 100 cm^3 soil with reproductive factor (*Rf*) and the mean fresh weight (ton/hectare ± standard deviation) of pea ('Bartesa' and 'Cher') after harvest from the entire plots. On field 1 plot size was 18 × 8m and on field 2 this was 12 × 8 m. Different letters per plot for yield indicate significant differences between plots (P ≤ 0.05).

Plot	Previous crop grown	Field 1 – Pea 'Bartesa'				Field 2 – Pea 'Bartesa'				Field 1 – Pea 'Cher'*		
		Pi	***Pf***	**Yield (ton/ha)**	***Rf***	***Pi***	***Pf***	**Yield (ton/ha)**	***Rf***	***Pi***	***Pf***	***Rf***
1	Tagetes	143	1110	23 ± 0.6 a	7.76	40	35	10 ± 0.1	0.87	181	791	4.37
2	Maize	255	503	16 ± 0.2 b	1.97	782	378	11 ± 0.1	0.48	617	469	0.76
3	Italian ryegrass	238	244	25 ± 0.3 a	1.02	168	49	11 ± 0.2	0.29	232	439	1.89

*no yield data available

For celeriac there was no correlation between yield and *Pi*. On field 2 lowest yield was found on the plots with the highest *Pf* but this was not the case on field 1. 'Celeriac 'Prinz' was a non-host to poor host based on *Pi*/*Pf* values from J2 in the field. *Rf* values ranged from 0.10 to 0.64 indicating a decline of the *M. chitwoodi* population on both fields.

Table 5 The mean initial *(Pi)*and final population (*Pf*) of *Meloidogyne chitwoodi* per 100 cm^3 soil with reproductive factor (*Rf*) and the mean fresh tuber weight (ton/hectare ± standard deviation)) of celeriac 'Prinz' after harvest from the entire plots. On field 1 plot size was 18 × 8m and on field 2 this was 12 × 8 m. Different letters per plot for yield indicate significant differences between plots (P ≤ 0.05).

Plot	Previous crop grown	Field 1 – Celeriac 'Prinz'				Field 2 – Celeriac 'Prinz'			
		Pi	*Pf*	Yield (ton/hectare)	*Rf*	*Pi*	*Pf*	Yield (ton/hectare)	*Rf*
1	Tagetes	68	7	75 ± 0.6	0.10	171	109	20 ± 0.5 b	0.64
2	Maize	971	361	68 ± 0.4	0.37	298	82	50 ± 1.7 a	0.27
3	Italian ryegrass	103	56	65 ± 0.5	0.54	121	61	47 ± 0.4 ab	0.50

Discussion

Based on our pot trials, all pea cultivars were considered to be a good host for *M. chitwoodi* with their high maximum population density (*M*) and maximum multiplication rate (*a*) values. With 652 DD_5 during the experiment *M. chitwoodi* was able to complete a generation as a first generation is completed between 500 and 600 DD_5 (Moens *et al*., 2009). Pea 'Electra' supported the highest population of *M. chitwoodi* followed by 'Cher'. In the field experiment, 'Cher' confirmed the results from the population dynamic model that was calculated for the pot test. With increasing *Pi*, the *Rf* reduced in the field. The degree-days (base 5°C) in the field was above 900. For 'Bartesia' we could see an increase of the *M. chitwoodi* population in field 1 whereas in field 2 the population declined, irrespective of the *Pi*. Both fields were located in the same area (5 km distance from each other) with identical climatologic conditions. Preliminary sampling in 2018 didn't reveal different nematode species composition in the fields (data not shown). Yield on field 2 was lower than on field 1 for both pea and celeriac. Pest and disease pressure could have been higher on field 2. For celeriac an infection with carrot fly was observed, but for pea no visual observation of pest and diseases was done. Soil fertility was not assessed but both fields were managed similarly in the year before sowing and planting. Soil compaction could also be a reason for less growth on field 2.

Our results confirm pea to be a good host plant for *M. chitwoodi* as shown earlier by Santo and Ponti (1985). A reduction in pea pod weight was observed as the nematode density increased in the pot trials. A yield reduction of 40 to 70% was calculated with the Seinhorst damage model but this could not be confirmed in the field experiments. However, less yield was obtained in field 1 for 'Bartesa' at the highest *Pi*. Santo and Ponti (1985) found that five out of six tested pea cultivars were tolerant to *M. chitwoodi* at *Pi* ranging from 125 to1250 eggs per 100 ml soil. This is in contradiction to our findings. The fitting of the Seinhorst damage model to our data was not optimal as reflected in the low R^2 values. For one pea cultivar we could not obtain standard errors for the parameters of the yield model. This can be explained because of the higher variation observed in the data and the resulting standard error. As pea is a self-pollinator, this variation cannot be explained by high genetic variability within cultivars. Pea is a legume that profits from legume-rhizobium symbiosis. Our pot experiments were done in heat sterilized soil and possibly the absence of rhizobium has influenced plant growth. In the nematode support tool best4soil (Best4Soil 2023) damage between 16 and 35% on pea is reported. Most likely, damage is cultivar dependent.

Celeriac 'Prinz' was an excellent host for *M. chitwoodi* with very high *M* and *a* values. Though in the pot trials at very high nematode densities of *Pi* > 100 J2 (100 cm³ soil)$^{-1}$ some observations were seen below the equilibrium line. This was a natural decline in the *Pf*, probably due to lack of space in the root system to keep up with the increasing nematode density (Schomaker & Been, 2013). A total of 3160 DD_5 was reached during the celeriac culture allowing multiple generations of *M. chitwoodi* to be produced. In the field experiment the *Rf* values were below one, which is according to our model given the higher *Pi* values in the field. Celeriac was sown in May and harvested in November. Wesemael and Moens (2008b) observed a decline of the *M. chitwoodi* population on a naturally infested field before fodder beet was harvested in November after initial strong increase of the population. Our analysis of the *Pf* from the soil samples was excluding eggs as we were not able to differentiate between nematodes eggs based on morphology. In the analysis of the roots of celeriac harvested from 1m^2 we found a substantial number of *M. chitwoodi* in the roots. Combined with reported winter survival of *M. chitwoodi* as eggs (Wesemael *et al*., 2006; Wesemael & Moens, 2008b) celeriac might lead to a high population of this nematode in the field. The damage threshold for celeriac was 11 J2 per 100 cm^3 soil and maximum 22% yield loss was observed. To our knowledge this is the first report of *M. chitwoodi* on celeriac. Kornobis (2004) found celeriac to be sensitive for *M. hapla* in Poland. On celery, *M. hapla*, *M. incognita* and *M. javanica* have been reported (Vovlas *et al.*, 2008; Melakeberhan & Wang, 2012).

From this study it is clear that growing pea and celeriac in *M. chitwoodi* infested fields can increase problems with this quarantine nematode. Differences in tolerance limit show the importance of cultivar selection for pea. The stubbles of peas have to be destroyed on the field after harvest to avoid further multiplication of nematodes. For celeriac, damage is limited but with its long field period, *M. chitwoodi* can have more than 1 generation. It is advisable to always include a non-host crop in a field rotation scheme after every cultivation of pea or celeriac in a field infested with *M. chitwoodi.* After pea a *M. chitwoodi* resistant cover crop can be grown. The late harvest of celeriac limits the options and black fallow during winter will be the best option. Most likely, following spring a peak in *M. chitwoodi* will occur as reported by Wesemael and Moens (2008b) due to hatching from overwintering eggs. Preferentially a non-host or tolerant crop is grown in the season after celeriac.

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Ethics declarations

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

References

Baermann, G. (1917). *A simple method for the detection of* Ankylostomum (nematode) *larvae in soil tests*. Batavia. Javasche Boekhandel & Drukkerij publishing.

Best4Soil (2023), Best4Soil - Nematode scheme > Nematode scheme (soilhealthtool.eu). Retrieved January 20, 2023, from https://www.best4soil.eu/

Brown, C., Mojtahedi, H., James, S., Novy, R., & Love. S. (2006). Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (*Meloidogyne chitwoodi)*. *American Journal of Potato Research* 83. 1-8. https://doi.org/10.1007/BF02869604.

Golden, A. M., O'Bannon, J., Santo, G., & Finley, A. (1980). Description and SEM observations of *Meloidogyne chitwoodi* n. sp.(Meloidogynidae). a root-knot nematode on potato in the Pacific Northwest. *Journal of Nematology* 12. 319-327.

Hendrickx, G. (1995). An automatic apparatus for extracting free-living nematode stages from soil. *Nematologica* 41. 308.

Dahl, W. J., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (*Pisum sativum* L.). *British Journal of Nutrition 108*. S3-S10. https://doi.org/10.1017/S0007114512000852.

Decraemer, W., & Hunt, D. J. (2013). Structure and classification. In: Perry. R.N. & Moens. M. (Eds). *Plant nematology.* Wallingford. UK. CABI Publishing. pp. 3-39. https://doi.org/10.1079/9781845930561.0003.

Griffin, G. (1989). Comparison of fumigant and nonfumigant nematicides for control of *Meloidogyne chitwoodi* on potato. *Journal of Nematology* 21. 640-644.

den Nijs, L., Brinkman, H., & van der Sommen, A. (2004). A Dutch contribution to knowledge on phytosanitary risk and host status of various crops for *Meloidogyne chitwoodi* Golden *et al*.. 1980 and *M. fallax* Karssen. 1996: an overview. *Nematology* 6. 303-312. https://doi.org/10.1163/1568541042360492.

Janssen, G. J., van Norel, A., Verkerk-Bakker, B., & Janssen, R. (1995). Resistance to *Meloidogyne chitwoodi. M. fallax* and *M. hapla* in wild tuber-bearing *Solanum* spp. *Euphytica* 92. 287-294. https://doi.org/10.1007/BF00037110.

Jaffee, B., & Muldoon, A. (1995). Susceptibility of root-knot and cyst nematodes to the nematode-trapping fungi *Monacrosporium ellipsosporum* and *M. cionopagum*. *Soil Biology and Biochemistry* 27. 1083-1090. https://doi.org/10.1016/0038-0717(94)00240-2.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., Wesemael, W. M. L. & Perry, R.N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular plant pathology* 14. 946-961. https://doi.org/10.1111/mpp.12057.

Karkanis, A., Ntatsi, G., Kontopoulou, C.-K., Pristeri, A., Bilalis, D., & Savvas, D. (2016). Field pea in European cropping systems: adaptability. biological nitrogen fixation and cultivation practices. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 44. 325-336. https://doi.org/10.15835/nbha44210618.

Kornobis, S. 2004. Diversity of *Meloidogyne hapla* Chitwood, 1949 population in Poland. *Russian journal of nematology* 12, 31-38.

Koshy, P., Eapen, S., & Rakesh, P. (2005). Nematode parasites of spices. condiments and medicinal plants. In: M. Luc, R. A. Sikora, & J. Bridge (Eds), *Plant parasitic nematodes in subtropical and tropical agriculture*(Ed. 2). Wallingford. UK. CABI Publishing. pp. 751-791. https://doi.org/10.1079/9780851997278.0751.

Melakeberhan, H., & Wang, W. 2012. Suitability of celery cultivars to infection by populations of *Meloidogyne hapla*. *Nematology* 14, 623-629. https://doi.org/10.1163/156854112X624186.

Miller, P. (1978). Reproduction. penetration. and pathogenicity of *Pratylenchus penetrans* on tobacco. vegetables. and cover crops. *Phytopathology* 68. 1502-1502.

Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* species–a diverse group of novel and important plant parasites. *Root-knot nematodes*. *1*. Wallingford. UK. CABI Publishing. pp. 483.

Nicol, J., Turner, S., Coyne, D. L., Nijs, L. d., Hockland, S., & Maafi, Z. T. (2011). Current nematode threats to world agriculture. In: J. Jones, G. Gheysen, & C. Fenoll (Eds), *Genomics and molecular genetics of plant-nematode interactions.* Springer. Dordrecht. pp. 21-43. https://doi.org/10.1007/978-94-007-0434-3_2.

Riga, E., Porter, L., Mojtahedi, H., & Erickson, D. (2008). *Pratylenchus neglectus. P. thornei.* and *Paratylenchus hamatus* nematodes causing yield reduction to dryland peas and lentils in Idaho. *Plant Disease* 92. 979-979. https://doi.org/10.1094/PDIS-92-6-0979B.

Santo, G., & Ponti, R. (1985). Host suitability and reaction of bean and pea cultivars to *Meloidogyne chitwoodi* and *M. hapla*. *Journal of Nematology* 17. 77-79.

Schomaker, C., & Been, T. (2013). Quantitative nematology and management. In: R. N. Perry, & M. Moens (Eds), *Plant nematology.* Wallingford. UK. CABI Publishing. pp.301-330.

Statbel.(2022). DBREF-L05-2021-TAB-A-NL.xlsx (live.com), Retrived January 18, 2023.

Vovlas, N., Lucarelli, G., Sasanelli, N., Troccoli, A., Papajova, I., Palomares-Rius, J. E., & Castillo, P. (2008). Pathogenicity and host-parasite relationships of the root-knot nematode *Meloidogyne incognita* on celery. *Plant Pathology* 57. 981-987. https://doi.org/10.1111/j.1365-3059.2008.01843.x.

Waeyenberge, L., Viaene, N., & Moens, M. (2009). Species-specific duplex PCR for the detection of *Pratylenchus penetrans. Nematology* 11. 847-857. https://doi.org/10.1163/156854109X428016.

Wesemael, W., Perry, R., & Moens, M. (2006). The influence of root diffusate and host age on hatching of the root-knot nematodes. *Meloidogyne chitwoodi* and *M. fallax*. *Nematology* 8. 895-902. https://doi.org/10.1163/156854106779799204.

Wesemael, W., Viaene, N., & Moens. M. (2011). Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology*. *13*(1). 3-16. https://doi.org/10.1163/138855410X526831.

Wesemael, W. M., & Moens, M. (2008a). Quality damage on carrots (*Daucus carota L.*) caused by the root-knot nematode *Meloidogyne chitwoodi. Nematology* 10. 261-270. https://doi.org/10.1163/156854108783476368.

Wesemael, W. M., & Moens, M. (2008b). Vertical distribution of the plant-parasitic nematode. *Meloidogyne chitwoodi*. under field crops. *European Journal of Plant Pathology* 120. 249-257. https://doi.org/10.1007/s10658-007-9213-x.

Wishart, J., Blok, V. C., Phillips, M. S., & Davies, K. G. (2004). *Pasteuria penetrans* and *P. nishizawae* attachment to *Meloidogyne chitwoodi. M. fallax* and *M. hapla*. *Nematology* 6. 507-510. https://doi.org/10.1163/1568541042665269.

Wishart, J., Phillips, M., & Blok, V. (2002). Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne chitwoodi. M. fallax.* and *M. hapla. Phytopathology* 92. 884-892. https://doi.org/10.1094/PHYTO.2002.92.8.884.