Host plant status and damage threshold of Spinach (*Spinacia oleracea*) for the temperate root-knot nematode *Meloidogyne chitwoodi*

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Summary

Problems with plant-parasitic nematodes remain a major challenge in most field vegetable crops worldwide. In agricultural fields in Belgium, these problems have in recent years increased. A major problem is the quarantine root-knot nematode *Meloidogyne chitwoodi*. The host plant status of the most commonly grown cultivars of spinach (*Spinacia oleracea*) and the damage threshold for *M. chitwoodi* were determined in a climate-controlled glasshouse and in a naturally infested field. The yield data with the final population densities fitted to Seinhorst's yield and population dynamic models respectively. The damage threshold values (*T*) for the relative fresh weight yield were 0.14, 1.08, 11.53 J2 per 100 cm^3 soil, minimum yield (*m*) of 0.84, 0.64, 0.52 and maximum yield (*Y*max) of 12.51, 12.44 and 9.60 for Gnu, Meerkat and Whale cultivars, respectively. The maximum multiplication rates (*a*) were 3.81, 2.38, and 2.72, while the maximum population densities (*M*) were 563, 916, and 922 J2 per 100 cm^3 soil for Gnu, Meerkat and Whale cultivars, respectively. *Meloidogyne chitwoodi* had an impact on the spinach yield. The higher the nematode density the lower the yield and vice versa. The tested cultivars were all hosts for *M. chitwoodi* but differences between cultivars occur. Cultivars Kolibri and Rhino were tested as the least sensitive cultivar for *M. chitwoodi* during the host screening test and bring opportunities for resistance breeding. This is the first report of spinach as a host for *M. chitwoodi*.

Key words: Yield, population dynamics, screening, resistance.

Introduction

Plant-parasitic nematodes remain a major challenge in most field vegetable crops worldwide. In Belgium, problems with nematodes increased in recent years. A survey conducted in the summer of 2015, revealed that among 50 vegetable growers 52% were already confronted with yield losses due to plant-parasitic nematodes (Van de Sande et al, 2015). These problems were found in vegetables, potatoes and sugar beets. From the companies surveyed, 30% were located in West Flanders, 24% in East Flanders, 30% in Antwerp and 16% in Limburg (Van de Sande et al, 2015). Wesemael (2007) stated that approximately 1/3 of the plots that were sampled to detect the presence of harmful nematodes (mainly *M. chitwoodi*, *M. fallax* and *P. penetrans*) before contracts are established for vegetable cultivation are rejected because of the nematode pressure. Analysis of seed potatoes for the presence of *P. penetrans* in 2008 (169 samples) and 2014 (174 samples) by the ILVO diagnostic center showed an increase in the infection rate from 63% in 2008 to 75% in 2014. The increasing problems can partly be explained by the sharp decrease in chemical soil disinfectants, more intensive use of machines and the misguided use of cover crops instead of fallow in the rotations (Molendijk & Mulder, 1996; Wesemael, 2007). For various vegetables specific knowledge about the relationship between nematode density and crop yield is lacking. Growers, advisers, suppliers, the processing industry and vegetable breeders all have specific questions and expect practical solutions that can contribute to the management of the nematode problem at company level.

The quarantine status of *M. chitwoodi* increases the economic consequences for the grower through the mandatory phytosanitary measures such as regular follow-up of plots and a ban on certain crops (FAVV, 2021). The control of *M. chitwoodi* with crop rotation is difficult due to its broad host plant spectrum (den Nijs et al., 2004; Jones et al., 2013; Moens & Perry, 2009; O'Bannon et al., 1982; Santo et al., 1980). Rotations that include crops with a short field period can overcome population build ups. Spinach (*Spinacia oleracea*) is an important crop in Belgian vegetable production. In 2020 spinach was grown on 4540 ha and the harvest of 94600 tonnes ranked Belgium as 10th on a global scale for spinach production (FAOSTAT, 2021). Under Belgian climatic conditions spinach can be grown from late Winter/early Spring till Autumn and the growing period is 55 to 70 days. In temperate agriculture, *Ditylenchus dipsaci*, *Heterodera schachtii*, *Pratylenchus penetrans* and *Meloidogyne hapla* have been associated with spinach (Castillo et al., 2007; Potter & Olthof, 1993). In several countries, *Meloidogyne* spp. are known to be one of the most damaging and common nematodes on spinach limiting the production (Basyony et al., 2020; Castillo & Jiménez-Díaz, 2003; Potter & Olthof, 1993). For the species *M. chitwoodi* no information on host plant status and damage is available.

The aim of this research was to determine the host plant status of the most commonly grown spinach cultivars 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

The population of *M. chitwoodi* (Smakt) used originated from a field in The Netherlands and was maintained as a pure culture at the Institute for Agricultural and Fisheries Research (ILVO). A mass culture of *M. chitwoodi* was done in closed containers on potato tubers *(Solanum tuberosum*) 'Bintje'. Potato tubers were thoroughly cleaned with tap water to remove soil particles and disinfected by submerging for a maximum of 5 minutes in a 5% solution of sodium hypochlorite (NaOCl). Afterwards the tubers were thoroughly rinsed with tap water to remove the disinfectant and kept to sprout at room temperature under daylight for about three to four weeks. Closed plastic containers (500 ml, 11 cm diameter., 8 cm height) were filled each with 200 g of sterilized (130°C, 4h) and dried white river sand and mixed with 30 ml sterile water. In each closed container, one sprouted potato tuber was placed on top of the soil and kept in the dark at 20°C for about 2 to 3 weeks to allow root development. Each container was inoculated with 2000 to 2500 second-stage juveniles (J2) of *M. chitwoodi* after root establishment. These inoculated closed containers were stored in the dark for 10 to 14 weeks at 20°C to allow nematode multiplication. The freshly hatched J2 used for both, pot and Cone tainers^{TM} experiments, were extracted from the potato roots by Baermann funnel technique (Baermann, 1917) in a mist chamber. The purity of the culture was confirmed molecularly with species-specific primers for *M. chitwoodi* as described by (Wishart et al., 2002).

Freshly hatched J2 (<24h) were tapped from the Baermann's funnels and allowed to settle for about 20 minutes. A vacuum pump (Vacuum brand, BVC 21 NT VARIO) was used to remove excess water to concentrate the nematode suspension. After homogenizing, the density of the J2 was determined by counting three subsamples of 1 ml. For the pot experiments inoculum densities following a log series 2^x (0, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512, J2/100 cm^3 soil) were prepared. For the screening test in Cone tainers™, 200 J2 per tube were needed.

POT EXPERIMENT ON DAMAGE THRESHOLD AND HOST PLANT STATUS

Seeds from the spinach cultivars used in the different experiments were kindly provided by Rijk Zwaan Zaadteelt en Zaadhandel B.V. (The Netherlands). An overview of the cultivars with characteristics is given in Table 1. Host plant status and damage threshold were determined in pot tests for 'Gnu', 'Whale' and 'Meerkat', three commonly used cultivars in Flanders. A total of 165 pots (2l) were filled with 1500 cm^3 heat sterilized (100°C for 16 hours) sandy loamy soil. Spinach seeds were sown (1 seed per pot, 55 pots per cultivar) and pots were completely randomized on raised benches in the greenhouse. The plants were watered three times per week upon requirements. After three weeks, when true leaves had developed, pots were inoculated with freshly hatched J2 of *M. chitwoodi* following a log series 2^x (0, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512, J2/100 cm^3 soil). Therefore, three holes of 5 cm depth were made around the plants to easily apply the nematodes with the aid of a pipette. One week after inoculation, 7g of a slow release fertilizer (N-P-K 16-9-12) was given. For each nematode density five replicates per spinach cultivar were used. Pot experiments took place in a greenhouse with controlled temperature conditions. The day and night temperatures were 20-23°C and 16 °C respectively with 12 hours of light. Eight weeks after sowing, the spinach reached its industrial required size and the experiment was terminated. The plants were carefully removed from the pots and both fresh leaf and root weight were measured. The soil from each pot was homogenized and a subsample of 200 cm^3 was taken. The roots were washed, weighed and chopped in small pieces of about 2 cm in length followed by maceration at high speed for one minute using a commercial Warring blender. Nematodes were extracted separately from soil subsamples and root samples with an automated zonal centrifuge technique (Hendrickx, 1995). Nematodes extracted from both the roots and soil samples were counted separately. Eggs extracted from the soil and root samples were considered to be *M. chitwoodi*. Nematode counts from the soil subsample were extrapolated to the total soil volume and the number of nematodes from the organic fraction were added to obtain the final density (*Pf*) in the pots.

FIELD EXPERIMENT ON DAMAGE THRESHOLD AND HOST PLANT STATUS

A naturally *M. chitwoodi* infested field located in the province of Limburg, Belgium was selected to evaluate the host plant status and damage threshold on spinach cultivar Gnu. Preliminary sampling was done in 2018 to confirm that *M. chitwoodi* was the only root-knot nematode species present. The nematode species was molecularly confirmed as described by (Wishart et al., 2002). The field was divided into 16 equal plots (18 × 8 m in size). In order to obtain a low, intermediate and high *M. chitwoodi* densities, respectively *Tagetes patula*, maize and Italian ryegrass were sown on the plots in 2018. Per plot a soil sample was taken before sowing spinach to determine the initial population (*Pi*). This was done by taking 60 soil cores from each plot, with the aid of a core (25 cm deep and 1.75 cm diameter) following a zigzag sampling pattern. Spinach was sown on three of these plots on 1st of April 2019 to 23th May 2019. Seven weeks after sowing, the spinach reached its required industrial size in the field. Commercial spinach yield per plot were determined by harvesting from 3 m^2. Spinach was also harvested from 1 m^2 plots coupled with intensive soil sampling (60 soil cores per 1 m^2) to link the spinach yield with nematode densities. From this 1 m^2, nematodes were extracted from both spinach roots and soil sample separately (Table 6).

HOST PLANT STATUS OF SPINACH CULTIVARS TO M. CHITWOODI

To obtain more knowledge on host plant status of spinach for *M. chitwoodi*, nine other cultivars 'Boa', 'Eagle ', 'Eland', 'Gorilla', 'Kolibri', 'Mouflon', 'Platypus', 'Puma' and 'Rhino' were screened (Table

1). Cone tainers™ (RLC4 type, Stuewe and Sons, USA) were filled with sieved (mesh 3.055 mm) sandy loamy soil sterilized at 100°C for 16 hours. In each Cone tainer™ one seed was sown. Tomato 'Marmande' was used as a control. For each cultivar 20 replicates were used. After seed germination and root establishment, each tube was inoculated with 200 freshly hatched J2 of *M. chitwoodi*. The plants were grown in a growing chamber at 18-23°C with 8 hours dark and 16 hours light. Plants were watered daily upon requirements. Eight weeks after nematode inoculation plants were carefully removed from the Cone tainers™ by submerging them in water. The roots of individual plants were carefully washed and kept in a 1-liter solution of 0.15 g phloxine B for 15 minutes to stain the gelatinous matrix of egg masses produced by *M. chitwoodi*. After staining, roots were rinsed in tap water and the number of egg masses per root system were counted and the average number of eggs per egg mass was determined using a binocular microscope. The soil was not considered and hence it was discarded after washing the roots.

STATISTICAL ANALYSIS AND MODELING

R studio, version 3.4.4 was used to analyse the data. The data obtained for the damage threshold, fitted the Seinhorst's model for yield (equation 1) as described by Schomaker and Been (2013). That for the host status fitted the equation (2) of sedentary nematodes with one generation per growing period of a crop (Seinhorst, 1967).

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

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

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

The weight of the spinach leaves expressed as a fraction of the weight obtained for spinach leaves at Pi = 0 J2/ 100cm³ soil, was described here as relative fresh shoot weight. The spinach leaves weights were then averaged over replicates for each nematode density used. The parameters T (= tolerance limit, damage threshold) for the maximum Pi density without noticeable damage, m for relative minimum yield, and $Y_{\max}$ for relative maximum yield were estimated for all measurements using equation (1). The two variables in this equation (1) are *Pi* which is the different nematode densities used and *Y* which is the weight of the plant parts or whole plant.

In order to estimate the maximum population density (*M*) and the maximum multiplication rate (*a*) the relation between *Pi* (initial population) and *Pf* (final population) was modelled using equation (2). The *Pf* obtained from each nematode density was log transformed, averaged over all replicates and back transformed at the end.

The coefficient of determination (R^2) used here expressed the goodness of fit of the model and was adjusted to degree of freedom. At $P = 0.05$, the least significant difference (LSD) was used to show the significant difference between spinach cultivars for all estimated parameters if necessary.

The number of egg masses of *M. chitwoodi* for the screening status test in Cone tainers™ and spinach yield on different plots under field conditions were subjected to analysis of variance (ANOVA) using a software program in R version 3.4.4. Differences among treatment means were compared using Fisher's least significant differences (LSD) at $P \leq 0.05$ and data were normalized by log transformation.

Nematode reproductive factor (*Rf*) for spinach under field conditions was equally calculated by using the formula $Rf = Pf/Pi$. Based on the *Rf* value the spinach 'Gnu' in field conditions could be classified under five different categories (Schomaker & Been, 2006) as follows: Non-host = (Rf <0.15), Poor host = (Rf < 1.0 ≥ 0.15), Maintenance host = (Rf ≤ 2.0 ≥1.0), Good host (Rf ≤ 4.0 ≥2.0) and Excellent host (Rf >4.0).

Results

YIELD AND DAMAGE THRESHOLD

All estimated parameters of the Seinhorst yield model with their standard errors (SE) and R^2 for the spinach marketable yield and root weight are summarized in Table 2 and 3 respectively. Maximum yield (Y_{max}) of cv. Whale (9.60 g) was significantly lower than that of cvs Meerkat (12.44 g) and Gnu (12.51 g).

Marketable yield

The relationship between the *Pi* and weight of the marketable parts (leaves) of the three spinach cultivars are represented in Fig. 1a. The damage threshold (*T*) for yield was lowest for cv. Gnu with 0.14 J2 per 100 cm^3 soil followed by cv. Meerkat (1.08 J2 per 100 cm^3 soil) and highest for cv. Whale with 11.53 J2 per 100 cm^3 soil. However, this difference was not significant due to the large standard error for cv. Whale (Table 2). Based on the relative minimum yields (*m*) the maximum yield loss for cv Gnu was 16%, for cv. Meerkat 36% and for cv. Whale 48%. For the minimum yield, a significant difference was observed between cvs. Gnu and Meerkat but not between cvs. Gnu and Whale due to the large standard error of cv. Whale. However, for a better comparison between the three cultivars, the same data was relatively plotted as seen in Fig. 1b.

Root weight

Apart from the marketable yield also the root weights (g) in relation to the different *Pi's* were studied (Fig. 2a). Galls were observed on the roots of the spinach cultivars in all inoculated pots. Root weight was analyzed with Seinhorst's equation (equation (1)) and results are shown in Table 3. With an increasing *Pi* the weight of the spinach roots increased (Fig. 2). Root weight increased most for 'Meerkat' (0.99 g), 'Gnu' (0.64 g) and the least was in 'Whale' (0.38 g) starting at thresholds of 3.10, 11.89 and 0.14 J2 per 100 cm^3 respectively (Table 3). Though the highest root weight was found in 'Meerkat' with a significant difference recognized at P=0.05 with 'Whale', minimum root weight was also observed on this 'Meerkat' at 1.67 g. This was followed by 'Gnu' (2.69 g) and then 'Whale' (3.16 g). However, the model (Table 3) fit was less good compared with those in Table 2 and 4. For a better comparison between the three cultivars, the same data was relatively plotted as seen in Fig. 2b.

Field analysis for the damage threshold

One cultivar of spinach 'Gnu' was used for the field experiment and no above ground symptoms due to nematode problems were observed. Though a negative correlation was seen in both nematodes in the spinach roots as well as in the soil (100 cm^3 soil) in relation to spinach yield as seen in table 4. Most *M. chitwoodi* were retrieved from the spinach roots and less from the soil (Table 4). There was no statistic significant difference found between plots with low and high initial population of *M. chitwoodi* and spinach yield (Table 6) at $P \leq 0.05$

POPULATION DYNAMICS

The relation between *Pi* and *Pf* for the three cultivars fitted well with the model for population dynamics (Equation (2)) for sedentary nematodes with one generation with R^2 values of 0.95, 0.98 and 0.94 for 'Whale', 'Gnu' and 'Meerkat' respectively (Table 5). All the fitted lines for the three cultivars could be observed above the equilibrium density line (*Pf = Pi*) as shown in Fig. 3. Both, maximum multiplication rate *a* as maximum population density *M* of 'Whale' were significantly different from 'Gnu' and 'Meerkat' (Table 5). The maximum multiplication rate (*a*) was highest for 'Whale' (3.81) but this cultivar supported the lowest maximum population density *M* (563 J2/ 100 cm^3 soil).

Population dynamics under field conditions

The population density of *M. chitwoodi* in the field reduced under spinach with an average reproductive factor of 0.72, 0.13 and 0.31 (Table 6) for the three plots. The field period of the spinach was 49 days. The *Pf* was determined based on the nematodes present in the mineral fraction.

SCREENING

On all the nine tested cultivars egg masses of *M. chitwoodi* were found but the numbers were significantly less compared to egg masses found on tomato (Table 7). The lowest number of egg masses was found on 'Rhino' and 'Kolibri'. Cultivar Kolibri had the lowest number of eggs per egg mass followed by 'Boa' and 'Rhino'. For 'Rhino' and 'Kolibri' 30% of the tested plants did not show any egg mass.

Discussion

All spinach seeds sown in both green house and field experiment could develop into spinach plants at all initial *M. chitwoodi* densities used. Reduction in plant growth was noticed when the population densities of *M. chitwoodi* increased, coupled with galling in the green house experiments. Very little to no galling was observed in the field experiments. In the field, the growing period was 49 days with 256 DD_5 which was not enough to develop severe galling. Knowledge about the damage threshold makes it possible to estimate whether damage will occur during the planned cultivation. With an increase in nematode population density the yield of spinach decreased for the three tested cultivars. In our pot experiments, the spinach fresh weight reduced with maximum 16, 36 and 48% with a tolerance limit for fresh weight recorded at *T* values of 0.14, 1.08 and 11.53 J2 per 100 cm^3 soil for 'Gnu', 'Meerkat' and 'Whale', respectively. The difference in tolerance limits and minimum yield shows that with choice of cultivar yield loss can be minimized or avoided. A prerequisite is knowledge about the initial population density. At a *Pi* below 12 J2 per 100 cm^3 cultivar Whale can be cultivated without yield loss whereas at higher *Pi* cultivar Gnu is a better choice with maximum 16% yield loss. This was confirmed in our field experiment where no difference in yield was seen for cultivar Gnu between plots with *Pi* 39 and 531 J2 per 100 cm^3. In one plot with *Pi* 91 J2 per 100 cm^3 a higher yield was obtained. This was probably due to the cultivation of Italian ryegrass that was preceding the spinach whereas in the other plots this was marigolds or maize. Positive N-effects of Italian ryegrass on subsequent yield have been reported for vegetables (Sorensen & Thorupkristensen, 1993) and rice (He et al., 2020).

The damage threshold for *M. chitwoodi* on the fresh areal weight of 'Gnu' (0.14 J2 per 100 cm^3), 'Meerkat' (1.08 J2 per 100 cm^3) and 'Whale' (11.53 J2 per 100 cm^3) were much lower compared to the threshold for *M. incognita* on spinach 'Symphony' (25 eggs/J2 per 100 cm^3) (Di Vito et al., 2004) and 'Yodha' (15.6 J2 per 100 cm^3) (Premachandra & Gowen, 2015). It is known that damage thresholds depend on species and population of the nematodes and are cultivar dependent. It seems spinach is more sensitive to *M. chitwoodi* than to *M. incognita*. On carrot 'Nerac' the damage threshold for *M. chitwoodi* was 34 to 50 J2 per 100 cm^3 depending on sowing density (Heve et al., 2015) whereas Wesemael and Moens (2008) reported damage on cultivars ABK and Amfine at Pi < 2 J2 per 100 cm^3. In wheat 'Nugaines', yield reduced up to 55% and 25% when the initial population of *M. chitwoodi* was 3 and 18 eggs per 100 cm^3 of soil in February and April, respectively (Nyczepir et al., 1984). For potato, thresholds of 0.4 (Santo et al., 1981) and 10 (Norshie et al., 2011) J2 per 100 cm^3 soil are reported. Our results confirm the low thresholds for *M. chitwoodi*.

The weight of the spinach roots increased with higher *Pi*. Symptoms on plant roots caused by root-knot nematodes can often be dramatic with the severity of galling depending on the host status of the plant or cultivar involved, nematode density and species of *Meloidogyne* involved. We observed more galls at higher *Pi* while the root length was similar which explains the increased weight.

Based on the results from the intensive sampling from 1 m² in the field plots, we observed a negative correlation between yield and total *M. chitwoodi* numbers in roots and soil. This finding was similar to previous studies showing that spinach growth is significantly reduced by *M. hapla* and *M. incognita* (Di Vito et al., 2004; Potter & Althof, 1974; Premachandra & Gowen, 2015; Sharma et al., 2001).,High numbers of nematodes where found in the spinach roots as well as in 100 cm³ soil samples from the 1 m² plots. Castillo and Jiménez-Díaz (2003) reported an incidence of *M. incognita* in 86% of the roots of spinach and 100% in the related soil samples comparable with our observations.

Host plant status

Both exponential and logistic Seinhorst models for population dynamics fitted to the data. The best fit was the exponential model for sedentary nematodes. All fitted lines were above the equilibrium line showing the three spinach cultivars were all host for *M. chitwoodi*. Cultivar Whale had a significantly higher multiplication rate (a) but also a significantly lower maximum population density (*M*) in the pot experiments compared to 'Gnu' and 'Meerkat'. The absolute root weight of 'Whale' was lower than that of 'Gnu' and 'Meerkat' which explains the lower maximum nematode density.

Nine different spinach cultivars were screened for multiplication of *M. chitwoodi* in Cone tainers™ and all were showing the formation of egg masses confirming spinach as a host plant for spinach. However, significantly less egg masses were found on spinach compared to tomato 'Marmande'. Differences between spinach cultivars could be observed. Cultivars Kolibri and Rhino had the lowest average number of egg masses per plant and the egg masses contained a relatively low number of eggs. For both cultivars no egg masses were seen on 31% of the tested plants. Cultivars Kolibri and Rhino can be recommended to the breeding sector for further work towards resistance against *M. chitwoodi*.

Based on the results of the *Pf/Pi* (*Rf*) values from our field experiment one would wrongly conclude spinach is not a host for *M. chitwoodi*. With low initial density, the population remained the same but with the intermediate and especially with a high *Pi* the population decreased. However, intensive sampling in 1 m^2 showed that *M. chitwoodi* was present in the spinach roots. In the field, spinach was grown for 49 days with a total degree days with base temperature 5°C (DD_5) (O'Bannon & Santo, 1984; Pinkerton et al., 1991) of 256 which was not enough for *M. chitwoodi* to complete a generation. In our pot experiments and screening test this was 652 DD_5. For potato and maize 555-740 DD_5 were reported for *M. chitwoodi* to complete its life cycle (Khan et al., 2014). Our pot experiments were done under optimum conditions for *M. chitwoodi* which was not the case in the field.

To our knowledge this is the first report of spinach cultivars being a host plant for *M. chitwoodi*. Spinach has a short cultivation period and can be grown in in Belgium in Spring and late Summer, beginning of Autumn. In general the field period is too short for multiplication of *M. chitwoodi* but caution is needed. Due to rising soil temperatures (global warming) during the spinach growing season, it is possible that *M. chitwoodi* will also complete a generation under field conditions. This can happen when spinach is sown late Summer when soil temperatures are higher. It is a common practice to leave the spinach stubbles on the field after harvest. On the roots, *M. chitwoodi* can continue its development and therefore it is important to destroy the stubbles immediately after harvest. As such, spinach can act as a trap crop. With the choice for selected cultivars both population build ups and damage can be avoided. Options for resistance breeding seem present. Taking into account these different aspects, spinach is a crop that can be included in rotation schemes to manage the quarantine root-knot nematode *M. chitwoodi*.

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Table 1. Overview on spinach cultivars (from www.rijkzwaan.nl).

Cultivar	Description
Kolibri	Fresh market Oval leaf shape, dark leaf color, thick leaf and strong against leaf diseases, Suitable for spring, autumn and winter.
Mouflon	Suitable for industry and grown in spring and summer.
Platypus	Fresh market Oval / pointed leaf shape, erected leaf position, thick leaf, good growing power, strong against leaf diseases. Suitable for spring, autumn and winter.
Eland	Variety suitable for industry and fresh market. For industry use in spring, fall and winter cultivation. Earliness between Meerkat and Dromedary.Coarse-leafed variety with good growing power, strong against leaf diseases
Gorilla	Industrial variety for spring, fall and winter cultivation. Earliness similar to Beaver and Gnu. Strong against Colletotrichum. Grows vigorous.
Boa	Industrial variety for spring and summer. Boa is 2-3 days faster than Puma. Slow shooting. Very productive. Very suitable for organic farming.
Rhino	Industry variety. Fast growing variety for spring, autumn and very late winter cultivation. Specially recommended for organic cultivation. The leaves are large and medium green. Resistant to cucumber mosaic virus.
Eagle	Industrial variety for spring and autumn cultivation. Forms large leaves.
Puma	Suitable for industry and is the variety for summer cultivation. Because Puma combines good vigor with slow shooting, the variety can be used very flexibly. It also shoots relatively slowly at fairly high temperatures. The leaves are thick, large and dark. Suitable for spring and summer.
Gnu	Industrial variety for spring, autumn and late winter cultivation. Earliness like Beaver and Gorilla. Oval leaf shape, good vigor, good petiole ratio, strong against shoots. The uniform stem length gives a low stem/leaf ratio.
Meerkat	Smooth-leaved spinach. Suitable for autumn and winter cultivation. A breed with earliness between Beaver and Sparrow. A variety with dark leaves and very uniform steel length, which gives a high yield and a low steel/leaf ratio.
Whale	Industrial variety.1 to 2 days later than Eagle. Whale forms slightly thicker and darker leaves than Eagle. Just like Eagle for spring and early fall cultivation.

Table 2. Parameter values from the Seinhorst's reduction yield equation , $Y = Y_{max}*(m + (1 - m) * 0.95^{(Pi-T)/T})$ for $Pi > T$ *and* y= 1 for $Y = 1$ for $Pi \leq T$ including their standard error (SE) and goodness of fit R^2 for the relation between *Pi* of *Meloidogyne chitwoodi* and spinach yield. The unit of Y_{max} is in gram and *T* is J2 /100 cm³ of soil. Parameter values were compared one on one.

Spinach cultivars	***m***	***T***	Y_{max}	SE_m	SE_T	SE_{Ymax}	R^2	**N**	**df**	$LSD._m$	$LSD._T$	$LSD._{Ymax}$
Gnu	0.84	0.14	12.51	0.05	2.35	0.75	0.60	11	9	-	-	-
Meerkat	0.64	1.08	12.44	0.08	0.81	0.86	0.69	11	9	0.19*|-	1.72 |-	2.40 |-
Whale	0.52	11.53	9.60	0.19	9.83	0.41	0.74	11	9	0.42 |0.18	20.65|64.78	1.79*|1.79*

*significantly different at P=0.05

Table 3. Parameter values from the Seinhorst's equation , $Y = Y_{\max}*(m + (1 - m) * 0.95^{(Pi-T)/T})$ for $Pi > T$ and $y= 1$ for $Y = 1$ for $Pi \leq T$ including their standard error (SE) and goodness of fit R^2 for the relation between *Pi* of *Meloidogyne chitwoodi* and spinach fresh root weight. The unit of $Y_{\max}$ is in gram and T is J2 /100 cm^3 of soil. Parameter values were compared one on one.

Spinach cultivars	m	T	Y_{max}	SE_m	SE_T	SE_{Ymax}	R^2	**N**	**df**	**LSD.$_m$**	**LSD.$_T$**	**LSD.$_{Ymax}$**
Gnu	2.69	11.89	0.64	1.60	23.12	0.21	0.29	11	9	-	-	-
Meerkat	1.67	3.10	0.99	0.51	6.20	0.22	0.18	11	9	3.54|-	50.28|-	0.65|-
Whale	3.16	0.14	0.38	2.12	0.09	0.26	0.57	11	9	5.59|6.55	48.57|32.44	0.70|0.35*

*significantly different at P=0.05

Table 1. The mean number of shoot weight (ton/hectare) and nematode counts (*M. chitwoodi*) per plots of spinach 'Gnu' from $1m^2$ (each plot was 18 x 8m in size), after harvest in roots and in $100cm^3$ soil.

Plot	***Pf* in roots**	***Pf* in soil**	**Yield (g)**
1	68	66.5	2883
2	326	439	2692
3	188	79.5	5026

Table 2. Parameter values of the population dynamics model for the relation between initial (*Pi*) and final population density (*Pf*) on a log scale. Multiplication rate (*a*) is dimensionless while, maximum population (*M*) is measured as *M. chitwoodi* (100 cm^3 soil)$^{-1}$. Parameter values were compared one on one.

Spinach cultivars	***a***	***M***	**SE**$_a$	**SE**$_M$	R^2	**N**	**df**	**LSD.*a***	**LSD.*M***
Whale	3.81	563	0.64	195	0.95	10	8	-	-
Gnu	2.38	916	0.22	374	0.98	10	8	1*|-	1738*|-
Meerkat	2.72	922	0.45	623	0.94	10	8	2*|1	2635*|1539.61

*significantly different at P=0.05

Table 3. The mean number of initial population *(Pi)*, final population of nematodes (*Pf*), reproductive factor (*Rf*) from 100cm³ soil and the mean number of shoot weight (ton/hectare ± standard deviation) after harvest from the entire plots (each plot was 18 x 8m in size) of spinach 'Gnu'. Different letters per plot for yield indicate significant differences between treatments ($P \leq 0.05$).

Plot	**Previous crop grown**	***Pi***	**P*f***	**Yield (ton/hectare)**	***Rf***
1	Tagetes patula	39	28	16.0 ± 2.23 **b**	0.718
2	Maize	531	70	17.5 ± 2.21 **b**	0.132
3	Italian ryegrass	91	28	26.7 ± 0.34 **a**	0.308

Table 4. Mean number of egg masses (mean ± Standard error), Percentage of plants with egg masses and eggs per egg mass of *Meloidogyne chitwoodi* 7 weeks after inoculation with 200 J2 per tested spinach cultivars (9 cultivars)with a tomato 'Marmande' as control.

Cultivar	**Mean number of egg masses per plant**	**Plant with egg masses (%)**	**Mean number of eggs per egg mass**
Kolibri	2.1±2.58 **a**	69	44.8
Mouflon	4.3±3.44 **b**	86	115.4
Platypus	3.5±4.58 **ab**	75	211.2
Eland	2.7±2.31 **ab**	82	208.8
Gorilla	3.0±3.02 **ab**	89	173.2
Boa	4.0±3.62 **ab**	93	93.4
Rhino	1.9±2.41 **a**	69	102.4
Eagle	3.5±2.45 **ab**	92	183.6
Puma	4.7±3.60 **b**	93	153.6
Tomato (control)	16.6±6.31 **c**	100	413.6

LEGENDS FOR FIGURES

Figure 1. The relationship between the initial population density (*Pi*) of *Meloidogyne chitwoodi*, on a log scale with **a**: Spinach yield (g) and **b**: Relative spinach yield at three spinach cultivars Whale, Gnu and Meerkat. The fitted line is according to Seinhorst equation, $Y = Y_{\max}*(m + (1 - m) * 0.95^{(Pi-T)/T})$ for $Pi > T$ *and y*= 1 for $Y = 1$ for $Pi \leq T$

Figure 2. The relationship between the initial population density (*Pi*) of *Meloidogyne chitwoodi*, on a log scale with **a**: Spinach roots (g) and **b**: Relative spinach root weight of three spinach cultivars Whale, Gnu and Meerkat. The fitted line is according to Seinhorst equation, $Y = Y_{\max}*(m + (1 - m) * 0.95^{(Pi-T)/T})$ for $Pi > T$ *and y*= 1 for $Y = 1$ for $Pi \leq T$

Figure 3. Relationship between initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne chitwoodi*, on a log scale for the three spinach cultivars Whale, Gnu and Meerkat. Fitted lines according to the equation: $Pf = Pf = M *(1 - e^{-a*Pi/M})$ for population dynamics and the Straight diagonal dashed line represent the population equilibrium line at $Pf = Pi$