Impact of cover crops on population density of the root-knot nematode *Meloidogyne chitwoodi*

Lirette M. TANING [1, 2, *], Eleni K. TAFES [3], Sander FLEERAKKERS [4], Louis LIPPENS [5], Ellen FORMESYN [6], Luc TIRRY [2] and Wim M.L. WESEMAEL [1, 2]

[1]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Burgemeester van Gansberghelaan 96, B-9820 Merelbeke, Belgium*

[2]*Ghent University, Laboratory of Agrozoology, Coupure links 653, B-9000 Ghent, Belgium*

[3]*Ghent University, Department of Biology, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium*

[4]*Proefstation voor de Groenteteelt (PSKW), Duffelsesteenweg 101, B-2860 Sint-Katelijne-Waver, Belgium*

[5]*Provinciaal Proefcentrum voor de Groenteteelt Oost-Vlaanderen vzw (PCG) Karreweg 6, B-9770 Kruisem, Belgium*

[6]*Inagro, Ieperseweg 87, B-8800 Rumbeke, Belgium*

Corresponding author, e-mail: Lirette.Taning@ilvo.vlaanderen.be

Summary - Management strategies applied to reduce *M. chitwoodi* nematode populations below economic damage threshold strongly depend on measures taken during the intercrop period. Therefore, this study evaluated the reproductive potentials of *M. chitwoodi* on different cover crop cultivars. Twenty two different cover crop cultivars were evaluated against *M. chitwoodi* at low and high initial inoculum density in a pot experiment under controlled conditions. Fallow was used as control. Based on the reproductive factor, the cover crops were classified under five different categories; Non-host, Poor host, Maintenance host, Good host and Excellent host. In this study, at both low (10 second-stage juveniles per 100 cm^3 soil) and high (100 second-stage juveniles per 100 cm^3 soil) initial inoculum density of *M. chitwoodi*, fodder radish 'Maximus', 'Contra', 'Dacapo', 'Defender', Italian ryegrass 'Meroa', rye 'Matador' and bird's foot trefoil 'Barguay', 'Franco', 'Lotar' were considered poor hosts reducing the *M. chitwoodi* population in the pot test based on *Rf* values. Field experiments were carried out which confirmed most of our results in the pot experiments. However, the field experiments showed that the rotation in which the cover crops are implemented are influenced by weather conditions, previous crop grown, their growing period and initial population densities. Based on our findings, selected non and poor host cover crops could be recommended for integrated management of *M. chitwoodi*.

Key words: Host status, reproductive factor, screening test, integrated nematode management, rotation

Introduction

Plant-parasitic nematodes are one of the important constraints in reducing both the quantity and quality of crops (Manzanilla-Lopez *et al*., 2004). Around 4000 species of plant-parasitic nematodes have been described (Decraemer & Hunt, 2013). Loss due to plant-parasitic nematodes in agriculture is globally estimated at about $US80 billion annually (Nicol *et al*., 2011). This number is an underestimation considering the unreported cases from farmers in tropic regions, who are unaware of symptoms of nematode attack due to their microscopic nature, atypical symptoms they cause and

their synergistic interaction with other pathogens (De Waele & Elsen, 2007). Most likely, also in temperate agriculture plant-parasitic nematodes and the damage they cause are often unknown. A survey conducted in 2012 resulted in a list of 10 globally important groups of plant-parasitic nematodes with root-knot nematode (*Meloidogyne* spp.), cyst nematode (*Heterodera* spp. and *Globodera* spp.) and root-lesion nematode (*Pratylenchus* spp.) as the first three in their respective order (Jones *et al*., 2013).

Root-knot nematodes have increased in importance in different parts of the world including Europe (Onkendi *et al*., 2014; Wesemael *et al*., 2011). In temperate climates the most economically important species are: *M. naasi*, *M. chitwoodi*, *M. hapla*, and *M. fallax*. In warmer conditions of southern Europe and in glasshouses, *M. arenaria*, *M. javanica* and *M. incognita* are the most common species (Moens & Perry, 2009; Wesemael *et al*., 2011). *Meloidogyne chitwoodi*, *M. fallax* and *M. enterolobii* are quarantine pests in Europe (Wesemael *et al*., 2011). *Meloidogyne chitwoodi* has a wide host range among several plant families (Golden *et al*., 1980; O' Bannon *et al*., 1982) making it difficult to manage this species.

Practices such as crop rotation, adjusting planting time, biological control with antagonists and physical methods like flooding, solarization and fallowing have so far been effective (Collange *et al*., 2011). However, most of these practices are only practical in small scale farming and are not sustainable in large scale production (McDonald & Nicol, 2005). The use of inorganic chemical pesticides has been an effective method (Haydock *et al*., 2013). Unfortunately, they are expensive, harmful to the environment, and the speed into which nematodes gain resistance renders them inefficient and ineffective in the long term. EU policy legally binds the reduction of the use and risk of chemical pesticides with 50% by 2030 and strongly promotes IPM. This increases the need for nematode resistant crops.

The best choices for the control of *Meloidogyne* nematodes are the use of crop rotation combined with resistant cultivars, non-hosts and fallow (Viaene *et al*., 2013). Given the wide host range of *M.*

chitwoodi this IPM strategy requires proper knowledge on host plant status of both cash crops and cover crops. It is known that populations of *M. chitwoodi* decrease markedly during winter under fallow (Noling & Becker, 1994; Pinkerton *et al*., 1991; Wesemael & Moens, 2008). Fallow is not supported in Belgium and cover crops are becoming more important. Cover crops are of economic importance to the soil and crop productivity. Cover crops that are grown during intermittent periods between cash crops, primarily to prevent soil erosion and increase soil quality, may help suppress nematode populations if properly selected (Nyczepir & Thomas, 2009). They can reduce plant-parasitic nematodes to below the economic damage threshold level and as such lead to an increase in the subsequent cash crop yield (Viaene & Abawi, 1998). Different crop cultivars of sorghum-sudan grass (*Sorghum bicolor* × *S. sudanense*), cowpea (*Vigna unguiculata*), sesame (*Sesamum indicum*), joint vetch (*Aeschynomene americana*), sunn-hemp (*Crotalaria juncea*), marigolds (*Tagetes* spp.), velvet bean (*Mucuna* spp.), hairy indigo (*Indigofera hirsuta*), castor (*Ricinus communis*), and grasses (*Poaceae*) which are commonly used as summer cover crops in Florida, can bring root-knot nematode populations below damage threshold levels (Gill & McSorley, 2011). Sudan grass, joint vetch, castor, velvet bean, cowpea and sorghum were effective cover crops to manage more than one species of root-knot nematodes (Gill & McSorley, 2011). For *M. chitwoodi* resistance has been reported in fodder radish (*Raphanus sativus*) (Teklu *et al*., 2014). Marigolds (*Tagetes patula*), English ryegrass (*Lolium perenne*), phacelia (*Phacelia tanacetifolia*), oilseed rape (*Brassica napus*), alfalfa (*Medicago sativa*) and common vetch (*Vicia sativa*) are non-hosts or poor hosts depending on the cultivar (Ferris *et al*., 1993; Best4soil, 2022).

Cover crops can also be used as trap crops for *Meloidogyne* species. An example is arugula *(Eruca sativa*) which is an effective trap crop for *M. hapla* (Melakeberhan *et al*., 2010). A trap crop allows J2 to enter the roots but due to antagonistic responses or destruction of the crop before the nematodes can complete their life cycle, their population can be reduced.

Regardless of all management strategies applied to reduce nematode populations below economic damage threshold, *M. chitwoodi* continues to be a big risk to the agricultural sector in Europe. It is difficult to suppress this nematode with a single management option. Knowledge on the host status of cover crops for *M. chitwoodi* is important as cover crops are more often used. Different cover crops showed to be sources of nematicidal compounds to suppress a number of plant-parasitic nematodes but many are also good hosts for *M. chitwoodi* (Thoden *et al*., 2011). Within a cover crop species, cultivars may vary in their suppressive effect to a particular nematode species. Therefore, this study evaluated the reproductive potential of *M. chitwoodi* on different cover crop cultivars sown in field or glasshouse conditions. The infectious process of *M. chitwoodi* on some cover crops was also studied to assess the possibility for further development of breeding programs for the management of *M. chitwoodi*. Knowledge on the development of *M. chitwoodi* would also facilitate the proper use of cover crops as trap crops.

Materials and methods

The pot and Cone tainers™ for host status and infectious process experiments took place at Flanders Research Institute for Agriculture, Fisheries and Food(ILVO), in Merelbeke, Belgium, in a temperature controlled glasshouse and growth chamber, respectively. A total of 22 cultivars of cover crop plants were used, with fallow and tomato used as a negative and positive control, respectively. General information regarding the cultivars are given in Table 1. Field experiments were done on two naturally infested fields with *M. chitwoodi* located in the province of Limburg in Belgium.

NEMATODE CULTURE

For the glasshouse experiments one *M. chitwoodi* population coming from a field in The Netherlands (Smakt population) was used. This population was cultured and maintained throughout the experimental period at Flanders Research Institute for Agriculture, Fisheries and Food (ILVO)), in Merelbeke, Belgium. *Meloidogyne chitwoodi* was maintained as pure culture on potato tubers (*Solanum tuberosum* 'Bintje'). The potato tubers used for the mass culturing were first thoroughly

washed with tap water to remove soil particles and then disinfected by submerging in a 5% NaOCl solution for 5 minutes. Afterwards the tubers were thoroughly rinsed with tap water to remove the disinfectant (NaOCl). The cleaned tubers were spread on tissue paper and left at room temperature for about 2 to 3 weeks to sprout. Plastic containers (10 cm diameter and 0.5 liter volume) were filled with 200 g of dried and sterilised river sand. To each container 30 ml distilled water was added and one sprouted potato tuber was placed into the sand. Containers were closed with a lid and then kept in a dark room for two weeks until tubers initiated enough roots in the sand. After the establishment of the root system, approximately 2000 second-stage juveniles (J2) of *M. chitwoodi* from the pure culture were inoculated in each closed container. The closed containers were kept at 20-22°C in a dark room for 10-14 weeks to allow nematode multiplication. After nematode multiplication, potato roots were chopped, and placed on Baermann funnel to extract nematodes (Baermann, 1917; Viaene *et al*., 2021). Only freshly hatched J2 (< 24 h) were used in the experiments. The purity of the nematode culture used for the pot experiments was confirmed molecularly as described by Wishart *et al*. (2002) and EFSA (2019).

PREPARATION OF SOIL AND FILLING POTS AND CONE TAINERS™

Sandy loamy soil was collected from a field at Merelbeke, Belgium and autoclaved at 100°C for 16 hours. The sterilised soil was later sieved with the aid of a 2.5 mm mesh sieve and mixed with river sand in the ratio of 1:1. Plastic pots (2 L, 16 cm diam.) were filled with 2000 cm^3 of this sterilised mixed soil and kept in the glasshouse for the host plant status experiment. Cone tainers™ (RLC4 type, Stuewe and Sons, USA) were used for the infectious process experiment. These Cone tainersTM were also filled with sterile soil each up to 1 cm from the top rim to ensure that the tubes do not overflow during watering. To maintain the soil moisture, the pots and tubes were watered before sowing the cover crops.

COVER CROPS

For the evaluation of the host status, different cover crops were sown individually in pots to which different nematode densities were added (see below). The surface area of the pot was selected as a function of the number of seeds sown (according to agricultural practices). The information of cover crops used and the relation of seed density with plant/cultivar is shown in Table 1. The first experiment for host status involved cultivars of Fodder radish, Black oat, Yellow mustard, Phacelia, Italian ryegrass, Rye, Summer oat while the second experiment was only with Bird's food trefoil cultivars.

PLANTING, MAINTENANCE AND NEMATODE INOCULATION

POT EXPERIMENTS FOR HOST PLANT STATUS

Fallow was used as control for the study of host status of different cover crops in pots. The pot experiments were done in a glasshouse with controlled conditions. Day length was 16 hours (20-23 °C) and night length was 8 hours (16 °C). Temperatures in the glasshouse were recorded per hour with a data logger (Testo) to calculate the amount of degree days (DD with 5 °C as base temperature for *M. chitwoodi*) during the period from inoculation to harvest. Plants were watered upon requirements to compensate evaporation and plant growth.

The inoculum was prepared, after determination of the nematode density of the stock suspension collected from the Baermann funnels. This was done by gently homogenizing the suspension, sub-sampling and counting nematodes under a stereo-microscope. Based on the density, the volumes needed for the different initial population densities (*Pi*) were calculated. A high (100 J2 per 100 cm^3) and a low (10 J2 per 100 cm^3) *Pi* density was used for the host plant status experiment in pots, with 5 replicates for each density used for the different cover crop plants. When plants were at 3-true-leaves stage, nematodes were inoculated in 3 holes (5 cm deep) with the aid of a glass pipette. Experimental setup for the host plant status in pots was a completely randomized design with five replicates per

crop/cultivar for each nematode density. The experiment was terminated 8 weeks after nematode inoculation.

CONE TAINERS™ EXPERIMENT FOR INFECTIOUS PROCESS.

The infectious study in Cone tainers™ had tomato 'Marmande' as a control. Cover crop cultivars used here were cultivars showing to be poor or maintenance host in the pot test. This experiment took place in a growth chamber, the temperature range was 18-20 °C with 16 hours of light and 8 hours of darkness. The inoculum was prepared as described above and plants (3-true-leaves stage) were inoculated with 200 J2 per Cone tainer™ with the aid of a glass pipette. For each cover crop cultivar 20 replicates were used. The infectious process was monitored at different time points, *viz.* 2, 7, 14 days post-inoculation (dpi); nematode reproduction was evaluated 8 weeks post inoculation.

NEMATODE EXTRACTION AND QUANTIFICATION.

POT EXPERIMENTS FOR HOST STATUS

The final population density of *M. chitwoodi* was estimated from the organic (roots) and mineral (soil) fraction per pot. After separation of mineral and organic fraction, root samples were washed and weighed for fresh weight. The cleaned roots were chopped in small pieces and macerated at high speed in a laboratory blender (Waring commercial) for 60 seconds. The blended roots were washed over a 850 µm sieve into a 1000 ml plastic beaker to remove residual root pieces. For mineral fraction, after homogenization of the soil, a subsample of 200 cm^3 of soil was taken and washed through a 850 µm sieve into a 1000 ml plastic beaker. The root and soil samples were separately subjected to the automated zonal centrifugation (AZC) (Hendrickx, 1995) to extract the nematodes. For the root sample, the AZC extracts 500 ml out of 1000 ml, hence to obtain the nematodes final population (*Pf*) in the whole root system, nematode counts were multiplied by factor 2. For the soil subsample, the AZC extracts 100 cm^3 out of 200 cm^3 of soil per pot, hence each soil sample nematode count represent

the quantity in 100 cm³ of soil. Nematode counts were used to calculate the final population (*Pf*) and reproductive factor (*Rf*) per plant/pot.

CONE TAINERS™ EXPERIMENT FOR INFECTIOUS PROCESS.

The penetration/infectious process was monitored at different time points viz. 2, 7, and 14 days post inoculation (dpi) through nematode staining in root tissue with the fuchsin acid method described by Byrd Jr *et al*. (1983). At each time point, 20 replicates per cultivar were used.

Eight weeks after inoculation, reproduction was assessed by counting the number of egg masses with the aid of Phloxine B (Sigma-Aldrich) that stains the gelatinous matrix as described by Daykin & Hussey (1985). This was done by removing the plants from the Cone tainers™ and carefully washing the root systems with tap water. These washed root systems were then soaked in Phloxine B solution (0.15-0.20 g per 1 l water) for 15-20 minutes. To determine the number of eggs per egg mass, eggs were extracted from the roots.. This was done by cutting the root system in to 1-2 cm pieces and macerating for 60 seconds at high speed in a laboratory blender (Waring commercial) in a 1% NaOCl solution. Afterwards, eggs were immediately extracted with the AZC as described above and the extracted eggs were counted with the aid of a binocular microscope.

FIELD EXPERIMENT

Two fields infested naturally with *M. chitwoodi* (molecularly confirmed as described by Wishart *et al*. (2002)) were used to examine the host status of some cover crop cultivars incorporated in a crop rotation scheme under field conditions. These fields were located in in Maaseik (N:51.114952, E:5.793017) and Kinrooi (N:51.1667, E:5.7333), Flanders-Belgium. Based on the soil analysis on both fields at a depth of 0 - 23 cm, the soil texture in Maaseik was a light sandy loam, with a soil pH of 6.4 and a carbon percentage (% C) of 1.64. In Kinrooi the texture was fine sand with a pH of 5.4 and % C of 1.40. The two fields were divided each into 18 equal plots, with the Maaseik field having equal plot

sizes of 18 × 8 m while on the Kinrooi field the plot size was 12 × 8 m. Three replicates (plots) were used per tested cover crop. To determine the initial population density (*Pi*) per plot, a soil sample was taken before sowing cover crops. This was done by taking 60 soil cores from each plot, with the aid of an auger (25 cm deep × 1.75 cm diam.) following a zigzag sampling pattern. Cover crops used here were fodder radish 'Terranova', 'Contra', 'Doublet' and 'Dacapo', yellow mustard 'Chacha', Italian ryegrass 'Meroa' and 'Melodia', black oat 'Pratex' and rye 'Matador' and 'Dukato'. Before the plots were prepared for the subsequent crop, soils samples were taken as described above to determine the final nematode population (*Pf*)..

DATA ANALYSIS

The data on host status and infectious process of cover crops with *M. chitwoodi* were subjected to analysis of variance (ANOVA) using a software program SAS. Differences among treatments (Mean comparison) were compared using Fisher's least significant differences (LSD) at $P \leq 0.05$ and data was normalized by log transformation (logx+1).

Nematode reproductive factor for each cover crop was equally calculated by using the formula (*Rf* = *Pf*/*Pi*). Based on the *Rf*, the cover crops were classified under five different categories (modified from Ferris *et al*., 1993) 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

HOST PLANT STATUS IN POTS

In this study, 818 DD_5 was calculated for the pot experiments for host status which was sufficient for *M. chitwoodi* to complete its life cycle (Moens *et al*., 2009).

The reproduction of *M. chitwoodi* varied with the cover crop (Tables 4 and 5). The results in Table 2 show that at a lower initial inoculum (low *Pi*) black oats 'Delux', 'Amazone', 'Pratex' and summer oat

'Effectiv' were excellent hosts. Yellow mustard 'Carnaval' was a good host. Fodder radish 'Doublet', yellow mustard 'Chacha', Italian ryegrass 'Fedra', rye 'Dukato' and summer oat 'Simphony' were maintenance hosts. Cover crops categorised as poor host were fodder radish 'Maximus', 'Contra', 'Dacapo', 'Defender', phacelia 'Angelia' and 'Natra', Italian ryegrass 'Meroa' and rye 'Matador'. At a higher initial inoculum (high *Pi*) only yellow mustard 'Carnaval' was an excellent host, black oats 'Pratex', 'Delux' and 'Amazone', summer oat 'Effectiv', yellow mustard 'Chacha', and fodder radish 'Doublet' were good hosts. Maintenance host were phacelia 'Angelia' and 'Natra', Rye 'Dukato' and Summer oat 'Simphony'. Fodder radish 'Maximus', 'Contra', 'Dacapo' and 'Defender' , Italian ryegrass 'Fedra' and 'Meroa' and rye 'Matador' were poor hosts.

A negative control (fallow) was subjected to the same inoculation levels but no nematodes were observed 8 weeks after the inoculation. No correlation was observed between nematode reproduction rate and the root weight of cover crops with high nematode numbers being equally recovered from cultivars with low root weight and higher root weight (Table 3). For most cultivars, an increase in root weight could be observed with a high *Pi* compared with low *Pi*.

When looking at the total number of nematodes in the pots per gram of root (Table 3) the significantly highest number was found in Summer oat 'Effectiv' at low *Pi* and in Yellow mustard 'Carnaval' at high *Pi*.

When comparing cultivars of the same cover crop, the *Pf* of *M. chitwoodi* on fodder radish 'Doublet' was significantly higher than the *Pf* on the other tested fodder radish cultivars at high *Pi* (Fig. 2). At low *Pi*, fodder radish 'Doublet' also showed highest *Pi* but here it was only significant compared with 'Maximus' and 'Contra' (Fig. 1). At high *Pi*, Italian ryegrass 'Meroa' had a significant higher *Pf* than 'Fedra'. For the other tested cover crops there was no significant difference between the tested cultivars.

In the second experiment for host plant status, Bird´s foot trefoil 'Barguay', 'Lotar' and 'Franco' were poor hosts while 'Bull' was a maintenance host. Differences between cultivars final population (*Pf*) were not significant at low initial *Pi* (Table 4 and 5). At high *Pi*, 'Franco' is a non-host while the other cultivars were poor host but also here differences were not significant.

INFECTIOUS PROCESS SCREENING TEST.

Based on the results from the pot experiments for host plant status, phacelia, Italian ryegrass and rye, which were poor or maintenance hosts, were further screened to monitor the penetration of *M. chitwoodi* at 2, 7 and 14 dpi. At each time point few nematodes were found inside the roots of all the cover crop cultivars being studied. At 14 dpi swollen stages of *M. chitwoodi* were not observed in roots. Tomato 'Marmande', used as the positive control, showed the highest number of penetrated nematodes at 2, 7 and 14 dpi (Fig. 3). The highest number of J2 penetration among the cover crops at time points 7 and 14 dpi was found in Italian ryegrass 'Fedra', while at 2 dpi rye 'Matador' showed the highest penetration of *M. chitwoodi* (Fig. 3). The average number of nematodes in the roots of rye 'Matador' decreased from 2 dpi (15 J2) to 7 dpi (2 J2) and 14 dpi (1 J2). For Italian ryegrass 'Fedra' and tomato 'Marmande' the number of nematodes inside the roots increased with time. At all-time points (dpi) the mean number of *M. chitwoodi* inside the roots of phacelia 'Angelia' and 'Natra' and Italian ryegrass 'Meroa' was low and not significantly different from each other (Fig. 3).

The multiplication of *M. chitwoodi* was studied at 8 weeks after inoculation (Table 6). The susceptible control tomato 'Marmande' had the highest mean number of egg masses per plant (88.5) and eggs per egg mass (107.42) and was significantly different from all tested cover crop cultivars. Phacelia 'Angelia' and 'Natra' and Italian ryegrass 'Fedra' showed very few egg masses being formed with a very low number of eggs per egg mass. Compared to these, Italian ryegrass 'Meroa' and rye 'Matador' had a significantly higher mean number of egg masses per plant. The number of eggs per egg mass found on rye was significantly higher compared to the other cover crops tested. Also, for rye,

egg masses were found on every tested plant. For Italian ryegrass 'Meroa' 90% of the plants showed egg masses whereas for 'Fedra' this was only for 20% the case.

HOST STATUS SCREENING TESTS UNDER FIELD CONDITIONS

Based on the results from the pot experiments and the availability of seeds, some cover crop cultivars were selected to be tested in a naturally *M. chitwoodi* infested field. There was a considerable variation in growth and/or germination of the different cover crops. Plots with greater cover crop coverage exceeding 90% (visual observation) were analyzed for their effect on the *M. chitwoodi* population (Table 7). The initial population ranged from low to high and the field period of the cover crops from 69 to 248 days. The degree-days with base temperature 5°C were calculated and are shown in Table 7. The tested fodder radish cultivars decreased the population of *M. chitwoodi* in the field except for one rotation where 'Dacapo' was sown after carrot 'Salto'. Yellow mustard 'Chacha' reduced the population but was sown at a very high *Pi*. Italian ryegrass 'Meroa' ranged from poor host to excellent host when sown after carrot 'Salto'; cv. Melodia was a maintenance host in the field. Phacelia 'Angelia' ranged from poor host to good host under field conditions. Black oat 'Pratex' was a poor or maintenance host unless it was sown after carrot 'Salto' when it was classified as excellent host. Rye showed to be at least a maintenance host and in 50% of the field trials it was an excellent host.

Discussion

Management of plant-parasitic nematodes with cover crops is challenging and strongly depends on the plant-parasitic nematode species present in the field. Our results show that for *M. chitwoodi* differences in host plant status between cultivars of the same cover crop exist and differential responses to the initial population density of *M. chitwoodi* occur. We investigated the impact of cover crop cultivars on different density levels of *M. chitwoodi* by comparing the nematode *Rf.* For 10 cultivars there was no difference in host plant status between the test at low *Pi* and high *Pi*. In 12 cases results for host status were different between high *Pi* and low *Pi*. Seven times a decrease in host plant

status was seen comparing high Pi with low Pi and five times an increase was seen.. It is known that at high population densities nematode multiplication is limited by competition and the total amount of food that the host can supply (Schomaker & Been, 2013). An increase in *Pf* at high *Pi* was seen in both cultivars of phacelia and yellow mustard and in one cultivar of fodder radish. For fodder radish and yellow mustard there was no difference in root mass between the pots with low and high *Pi*. Both phacelia cultivars had a higher root mass in the pots with high *Pi* but also a higher *Pf* per gram of root. Fodder radish 'Doublet' has resistance against *M. chitwoodi*. However, in our pot experiment it was a maintenance host at low *Pi* and a good host at high *Pi*. In the field experiment, with a high *Pi*, it was classified as poor host. The DD_5 in the field was lower compared to the pot test but high enough to allow a new generation of *M. chitwoodi* to be formed. The countings from the field samples could be an underestimation of the actual population because eggs were not considered. It is also possible that Meloidogyne resistance was broken in the pot test due to high nematode pressure as reported for the *Mi* gene in tomato (Padilla-Hurtado *et al*., 2021, Maleita *et al*., 2012). It is clear from our pot test that a different inoculum density can render different results. To overcome this, host plant status studies can be done by developing a population dynamics model with a range of initial inoculum densities as described by Seinhorst (Schomaker & Been, 2013). The latter will provide a better view on host plant status but is time consuming and costly. For cash crops, where population dynamic studies can be combined with damage threshold studies this can be justified. For cover crops, a screening for egg mass production will allow a faster and cheaper initial judgement on the potential of *M. chitwoodi* to multiply on the crop. This can be complimented with a pot test in which a low and high *Pi* are used to elucidate on population density influences. Teklu *et al*. (2014) report that for fodder radish, testing at a single population density is possible when relative susceptibility is stable.

In this study, at both low and high initial inoculum density of *M. chitwoodi*, fodder radish 'Maximus', 'Contra', 'Dacapo', 'Defender', Italian ryegrass 'Meroa', rye 'Matador' and bird's foot trefoil 'Barguay', 'Franco', 'Lotar' were considered poor hosts reducing the *M. chitwoodi* population in the pot test based on *Rf* values. Our results confirm earlier findings of Teklu *et al*. (2014) for fodder radish. Also in the

field experiments the tested fodder radish cultivars were classified as poor or non-host. Except for one plot were fodder radish 'Dacapo' was sown after carrots. This was also seen for Italian ryegrass 'Meroa' that was a poor host in the field experiments except when sown after carrots and for black oat. Most likely, after carrots a proportion of the *M. chitwoodi* population remained in the field as eggs from which J2 hatched the following spring. It is known that *M. chitwoodi* populations increase in Spring when soil temperatures increase probably due to hatching from overwintering eggs (Pinkerton *et al*., 1991, Wesemael & Moens, 2008). The presence of these eggs depends on the condition of the host plant and seem to increase on senescing plants (Wesemael *et al*., 2006). In our field experiment, the carrots were not harvested but were ploughed in before the cover crops were sown. For rye, the field experiments showed that this cover crop will increase the *M. chitwoodi* population whereas from the pot experiment where rye was a maintenance or poor host, we would not expect this. Ferris *et al*. (1993) showed in a pot test that rye was a maintenance host for *M. chitwoodi.* When we further examined the formation of egg masses on rye we could see that egg masses were found in all the tested plants of 'Matador' confirming its potential to multiply *M. chitwoodi*. For black oat the results from the field classified this crop as poor host or maintenance host except when sown after carrot. In the pot test black oat was a good to excellent host. Yellow mustard 'Chacha' was a poor host in the field experiment but maintenance to good host in the pot experiment. Phacelia 'Angelia' ranged from poor host to good host in the field experiments whereas in the pot test it was a poor to maintenance host. Also few egg masses were found and few J2 penetrated the roots. Viaene and Abawi (1998) classified phacelia as maintenance host having a reproductive factor (*Rf*) close to 1 for *M. hapla*.

Bird's foot trefoil showed to be poor host in the pot tests. Unfortunately we could not obtain results from the field as due to slow germination and growth of the bird's foot trefoil, the plots were overgrown by weeds. Bird's foot trefoil was reported as maintenance host for *M. incognita* (Moye *et al*., 2018) and a non-host for *M. graminicola* (Negretti *et al*., 2014).

In this research pot experiments were not repeated in time but complemented with studies on nematode development and field trials. It is clear from our results that conclusions from pot experiments are not always confirmed under field conditions. In the field several biotic and abiotic factors will influence the results. Apart from variability in population densities within the field and growing period, we have seen that the rotation in which cover crops are implemented can play a vital role to determine whether they will decrease the *M. chitwoodi* population. Ferris *et al*. (1993) indicated that rye, oat, barely, wheat and white lupine were classified as a maintenance host for *M. chitwoodi.* They support the reproduction on the same level without increasing or reducing, but there was a considerable decline in the population when it was used following a fallow period. This makes it difficult to give proper advice to farmers. A critical evaluation of available scientific data is needed.

Based on our combined results from pot and field experiments we can conclude that fodder radish cultivars with *M. chitwoodi* resistance gave very good results in reducing the population. In field conditions, a short cultivation before the summer (April to July) results in a 70 to 80% drop in the population. Classic use in the autumn and winter gives a decrease of 30 to 50%. The largest decrease (up to 100%) can be achieved when a spring crop with a resistant fodder radish (April to July) is followed by black fallow during the summer (July) and an autumn crop with resistant fodder radish (sowing in September). At high *M. chitwoodi* pressure, fodder radish may lag behind in growth. After the cultivation of (late) carrots (harvest in July or August), sowing a *M. chitwoodi* resistant fodder radish in the autumn did not decrease the population. Phacelia is a maintenance host plant for *M. chitwoodi*. With a short cultivation period (<70 days) during the period April to July, phacelia can reduce the population as a trap crop. With a longer field period, phacelia will maintain *M. chitwoodi* or allow the population to increase to a limited extent. Yellow mustard is a host plant for *M. chitwoodi* and will increase the population. With a short cultivation period (<70 days) during the period April - July, yellow mustard can be used as a catch crop. It is then important to destroy the yellow mustard before *M. chitwoodi* can lay new eggs. The host plant status of Italian ryegrass for *M. chitwoodi* is cultivar dependent. There are cultivars that increase, maintain or decrease the population. This is

independent of the cultivation time and the period of the year. When sowing in spring, the chance of weeds is greater, which increases the chance of multiplication of *M. chitwoodi* on these weeds. After the cultivation of carrots followed by the cultivation of Italian ryegrass during the autumn and winter, we saw a strong increase in the population in the following spring. Black oats are a host plant for *M. chitwoodi* and will maintain or increase the population. With a short cultivation period (<70 days) during the period May - July, black oats can be used as a catch crop. Rye is a good host for *M. chitwoodi*. When a cover crop is sown late in the season, Italian ryegrass is a better choice than rye. If rye is sown anyway, it is recommended to destroy it early in the spring (February), especially after a mild winter, to prevent the formation of eggs. Bird's foot trefoil is not a host plant for *M. chitwoodi* and will cause the population to decline (natural decline). However, the cultivation is very sensitive to weeds and this can nullify the beneficial effect. Weed control is necessary. The infectious screening test in Cone tainers™ gave us more information on the penetration, development and multiplication of this nematode creating more possibility for future development in breeding programs for the management of this nematode.

Acknowledgement

This study was funded by Flanders Innovation & Entrepreneurship (vlaio, contract HBC.2016.0770) and supported by the Belgian Plant Protection Service (FAVV) (agreement 10-ILVOCRA-Planten).

References

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

Best4soil (2022). Data base of nematodes. Best4Soil - Nematode scheme > Nematode scheme (soilhealthtool.eu), accessed 17/10/2022.

Byrd Jr, D., Kirkpatrick, T. & Barker, K. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15, 142-143.

Collange, B., Navarrete, M., Peyre, G., Mateille, T. & Tchamitchian, M. (2011). Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. *Crop protection* 30, 1251-1262. DOI:org/10.1016/j.cropro.2011.04.016.

De Waele, D. & Elsen, A. (2007). Challenges in tropical plant nematology. *Annual Review of Phytopathology* 45, 457-485. DOI:10.1146/annurev.phyto.45.062806.094438.

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. DOI:10.1079/9781845930561.0003.

EFSA (European Food Safety Authority)., den Nijs, L., Camilleri, M., Diakaki, M., Schenk, M., & Vos, S. (2019). Pest survey card on *Meloidogyne chitwoodi* and *Meloidogyne fallax*. EFSA Supporting Publications 16, 1572E. DOI:org/10.2903/sp.efsa.2019.EN-1572

Ferris, H., Carlson, H., Viglierchio, D., Westerdahl, B., Wu, F., Anderson, C., Juurma, A. & Kirby, D. (1993). Host status of selected crops to *Meloidogyne chitwoodi*. *Journal of Nematology* 25, 849-857.

Gill, H. K. & McSorley, R. (2011). Cover crops for managing root-knot nematodes. EDIS, 2011(7).

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.

Haydock, P., Woods, S., Grove, I. & Hare, M. (2013). Chemical control of nematodes. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology* . Wallingford, UK, CABI Publishing, pp. 459-479. DOI:10.1079/9781780641515.0459.

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

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular biology and evolution* 23, 1792-1800. DOI:org/10.1093/molbev/msl044.

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. DOI:org/10.1111/mpp.12057.

Maleita, C.M.N., Curtis, R.H.C., Powers, S.J. & Abrantes, I.M.D. (2012). Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction, and growth of tomato genotypes. *Phytopathologia Mediterranea* 51, 566-576.

Manzanilla-Lopez, R., Evans, K. & Bridge, J. (2004). Plant diseases caused by nematodes. In: Z. X. Chen, S. Y. Chen, & D. W. Dickson (Eds.). *Nematology—Advances and perspectives, Volume II: Nematode management and utilization.* Cambridge, MA, CABI Publishing, pp. 637-716.

McDonald, A. & Nicol, J. (2005). Nematode parasites of cereals. In: Luc, M.; Sikora, R. A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford, UK, CABI Publishing, pp. 131-191. DOI:10.1079/9780851997278.0131.

Melakeberhan, H., Kravchenko, A., Dahl, J. & Warncke, D. (2010). Effects of soil types and *Meloidogyne hapla* on the multi-purpose uses of arugula (*Eruca sativa*). *Nematology* 12, 115-120. DOI: org/10.1163/156854109X456853

Moens, M. & Perry, R. N. (2009). Migratory plant endoparasitic nematodes: a group rich in contrasts and divergence. *Annual Review of Phytopathology* 47, 313-332. DOI: 10.1146/annurev-phyto-080508–081846.

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

Moye, Jr., H.H., Xiang, N., Groover, W., Lawrence, K. & van Santen, E. (2018). First report of the root-knot nematode (*Meloidogyne incognita*) on Birdsfoot trefoil (*Lotus corniculatus L.*) in the Southern United States. *Plant Disease* 102, 684-684. DOI: 10.1094/PDIS-08-17-1299-PDN

Negretti, R.R.D., Manica-Berto, R., Agostinetto, D., Thuermer, L. & Gomes, C.B. (2014). Host suitability of weeds and forage species to root-knot nematode *Meloidogyne graminicola* as a function of irrigation management. *Planta daninha* 32, 555-561. DOI: 10.1590/S0100-83582014000300011

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

Noling, J. W. & Becker, J. O. (1994). The challenge of research and extension to define and implement alternatives to methyl bromide. *Journal of Nematology* 26, 573-586.

Nyczepir, A. P. & Thomas, S. H. (2009). 18 Current and future management strategies in Intensive crop production systems. In: Moens, M., Perry, R. N., & Starr, J. L. (Eds). *Root-knot nematodes*. Wallingford, UK, CABI Publishing, pp.412-435.

O'Bannon, J., Santo, G. & Nyczepir, A. (1982). Host range of the Columbia root-knot nematode. *Plant Disease*, 66, 1045-1048. DOI: 10.1094/PD-66-1045.

Onkendi, E. M., Kariuki, G. M., Marais, M. & Moleleki, L. N. (2014). The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: a review. *Plant Pathology* 63, 727-737. DOI:org/10.1111/ppa.12202.

Padilla-Hurtado, B., Morillo-Coronado, Y., Tarapues, S., Burbano, S., Soto-Suárez, M., Urrea, R. & Ceballos-Aguirre, N. (2022). Evaluation of root-knot nematodes (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene Mi-1. *Chilean Journal of Agricultural Research* 82, 157-166. DOI: 10.4067/S0718-58392022000100157.

Pinkerton, J. N., Santo, G. S. & Mojtahedi, H. (1991). Population dynamics of *Meloidogyne chitwoodi* on Russet Burbank potatoes in relation to degree-day accumulation. *Journal of Nematology*, 23, 283-290.

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

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

Teklu, M. G., Schomaker, C. H. & Been, T. H. (2014). Relative susceptibilities of five fodder radish varieties (*Raphanus sativus* var. oleiformis) to *Meloidogyne chitwoodi*. *Nematology*, *16*(5), 577-590. DOI:org/10.1163/15685411-00002789.

Thoden, T. C., Korthals, G. W. & Termorshuizen, A.J. (2011). Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? *Nematology* 13, 133-153. DOI:10.1163/138855410X541834

Viaene, N., Hallmann, J. & Molendijk, L. P. (2021). Methods for nematode extraction. In: Perry, R. N., Hunt, D. H & Subbotin, S. A. (Eds). *Techniques for work with plant and soil nematodes*. Wallingford. UK. CABI Publishing. pp. 12-41

Viaene, N., Coyne, D. L. & Davies, K. G. (2013). Biological and cultural management. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*(Ed. 2). Wallingford, UK, CAB International, pp. 383-410. DOI:10.1079/9781780641515.0383.

Viaene, N. M. & Abawi, G. S. (1998). Management of *Meloidogyne hapla* on lettuce in organic soil with sudangrass as a cover crop. *Plant Disease* 82, 945-952. DOI:org/10.1094/PDIS.1998.82.8.945.

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

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

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. DOI:org/10.1094/PHYTO.2002.92.8.884.

Table 1: Information on cover crops, seed rate and number of seeds per pot.

Common name	Scientific name	Cultivar	1000 seed weight (g)	Seed source	Seed density (kg/ha)	Seed density per/pot (g/0.02m²)	Number of seed per pot
Bird's foot trefoil	*Lotus corniculatus*	Bull	140	Freudeberger, Germany	10	0.02	14
		Barguay	1.20	Barenbrug, Netherlands			
		Lotar	1.25	Oseva Seeds, Czech Republic			
		Franco	1.13	Entecra, Italy			
Phacelia	*Phacelia tanacetifolia*	Angelia	1.8	AVEVE, Belgium	10	0.024	16
		Natra	1.5	AVEVE, Belgium			
Fodder radish	*Raphanus sativus*	Contra	13.2	Petersen, Germany	40	0.08	4
		Dacapo	11.1	Petersen, Germany			
		Defender	11.40	Petersen, Germany			
		Doublet	11.60	Joordens, Netherlands			
		Maximus	12.40	ILVO, Belgium			
Italian ryegrass	*Lolium multiflorum*	Fedra	4.47	ILVO, Belgium	15	0.03	8
		Meroa	4.84	ILVO, Belgium			
Black oats	*Avena strigosa*	Delux	15.22	AVEVE, Belgium	40	0.08	5
		Amazone	15.32	Limagrain, Belgium			
		Pratex	15.28	AVEVE, Belgium			
Rye	*Secale cereale*	Dukato	36.52	AVEVE, Belgium	70	0.14	4
		Matador	36.41	AVEVE, Belgium			
Summer oats	*Avena sativa*	Effectiv	37.37	AVEVE, Belgium	100	0.2	5
		Simphony	37.23	AVEVE, Belgium			
Yellow mustard	*Sinapis alba*	Chacha	5.3	AVEVE, Belgium	20	0.04	8
		Carnaval	5.49	Limagrain, Belgium			

Table 2: Reproductive factor (*Rf* ± standard error) of *Meloidogyne chitwoodi* (Smakt population) on different cover crops 8 weeks after inoculation with 10 J2/100 cm³ (low *Pi*) and 100 J2/100 cm³ (high *Pi*). A negative control (fallow) was subjected to the same inoculation. Different letters per column indicate significant differences between cultivars ($P \leq 0.05$)..

Crop	Cultivar Name	Low *Pi*		High *Pi*	
		Rf	**Host status**	***Rf***	**Host status**
Fodder radish	Maximus	0.45 ± 0.09 ^{c}	Poor host	0.37 ± 0.07 ^{d}	Poor host
	Contra	0.40 ± 0.09 ^{c}	Poor host	0.48 ± 0.08 ^{d}	Poor host
	Dacapo	0.74 ± 0.18 ^{c}	Poor host	0.60 ± 0.17 ^{d}	Poor host
	Defender	0.74 ± 0,17 ^{c}	Poor host	0.90 ± 0.41 ^{cd}	Poor host
	Doublet	1.44 ± 0.20 ^{bc}	Maintenance host	2.47 ± 0.48 ^{bcd}	Good host
Black oat	Delux	4.33 ± 2.06 ^{abc}	Excellent host	2.28 ± 0.95 ^{bcd}	Good host
	Amazone	5.57 ± 1.59 ^{ab}	Excellent host	2.08 ± 1.60 ^{bcd}	Good host
	Pratex	4.58 ± 2.18 ^{abc}	Excellent host	2.46 ± 0.72 ^{bcd}	Good host
Yellow mustard	Chacha	1.74 ± 0.33 ^{bc}	Maintenance host	3.65 ± 0.89 ^{ab}	Good host
	Carnaval	2.09 ± 0.60 ^{bc}	Good host	4.65 ± 0.82 ^{a}	Excellent host

Table 2 (Continued)

Crop	Cultivar Name	Low *Pi*		High *Pi*	
		Rf	**Host status**	***Rf***	**Host status**
Phacelia	Angelia	0.69 ± 0.28 ^{c}	Poor host	1.32 ± 0.31 ^{cd}	Maintenance host
	Natra	0.33 ± 0.14 ^{c}	Poor host	1.58 ± 0.25 ^{bcd}	Maintenance host
Italian ryegrass	Fedra	1.88 ± 0.55 ^{bc}	Maintenance host	0.46 ± 0.05 ^{d}	Poor host
	Meroa	0.72 ± 0.40 ^{c}	Poor host	0.67 ± 0.06 ^{d}	Poor host
Rye	Dukato	1.12 ± 0.32 ^{c}	Maintenance host	1.04 ± 0.11 ^{cd}	Maintenance host
	Matador	0.29 ± 0.18 ^{c}	Poor host	0.55 ± 0.15 ^{d}	Poor host
Summer oat	Simphony	1.88 ± 0.54 ^{bc}	Maintenance host	1.98 ± 0.44 ^{bcd}	Maintenance host
	Effectiv	7.35 ± 4.22 ^{a}	Excellent host	3.01 ± 0.50 ^{abc}	Good host
Fallow		0.00 ± 0.00		0.00 ± 0.00	

Table 3: Final population density of *Meloidogyne chitwoodi* per gram of roots (*Pf* ± standard error), root weight (mean ± standard error) and final nematode population (*Pf* ± standard error) on different cover crops extracted 8 weeks after inoculation with 10 J2/100 cm³ (low *Pi*) or 100 J2/100 cm³ (high *Pi*). Different letters per column for root weight (g) and *Pf* per gram of roots indicate significant differences between cultivars per cover crops ($P \leq 0.05$). While the Different letters per column for final population (*Pf*) indicate significant differences between cultivars ($P \leq 0.05$)..

Crop	Cultivar Name	Low *Pi*			High *Pi*		
		Root Weight (g)	Final population (*Pf*)	*Pf* per gram of roots	Root Weight (g)	Final population (*Pf*)	*Pf* per gram of roots
Fodder radish	Maximus	22.7 ± 2.45 ^{b}	90.0 ± 18.86 ^{c}	4.2 ± 1.05 ^{a}	33.4 ± 3.86 ^{a}	749.6 ± 132.10 ^{e}	23.9 ± 4.32 ^{b}
	Contra	20.5 ± 2.95 ^{b}	80.8 ± 18.23 ^{c}	4.6 ± 1.60 ^{a}	30.5 ± 4.92 ^{a}	953.1 ± 157.39 ^{de}	38.7 ± 12.82 ^{b}
	Dacapo	18.6 ± 1.97 ^{b}	148.0 ± 35.03 ^{c}	8.5 ± 2.36 ^{a}	34.6 ± 2.80 ^{a}	1195.2 ± 343.69 ^{de}	32.8 ± 6.11 ^{b}
	Defender	18.7 ± 1.55 ^{b}	147.2 ± 34.21 ^{c}	8.7 ± 2.98 ^{a}	31.5 ± 2.33 ^{a}	1800.2 ± 811.40 ^{de}	61.8 ± 30.96 ^{b}
	Doublet	34.2 ± 2.48 ^{a}	288.8 ± 40.27 ^{bc}	8.8 ± 1.69 ^{a}	33.5 ± 4.80 ^{a}	4934.4 ± 952.56 ^{bcd}	172.5 ± 56.97 ^{a}
Black oat	Delux	54.8 ± 4.87 ^{a}	866.4 ± 411.51 ^{abc}	15.6 ± 6.47 ^{a}	106.2 ± 5.49 ^{a}	4554.2 ± 1900.93 ^{bcde}	43.6 ± 17.59 ^{a}
	Amazone	40.4 ± 5.30 ^{a}	1113.2 ± 317.15 ^{ab}	28.8 ± 9.62 ^{a}	85.6 ± 10.46 ^{ab}	4152.0 ± 3197.76 ^{bcde}	50.5 ± 39.78 ^{a}
	Pratex	44.4 ± 4.39 ^{a}	916.0 ± 435.02 ^{abc}	24.0 ± 11.79 ^{a}	67.8 ± 6.61 ^{a}	4924.8 ± 1431.13 ^{bcd}	81.1 ± 26.12 ^{a}
Yellow mustard	Chacha	18.6 ± 2.83 ^{a}	347.2 ± 65.08 ^{bc}	21.1 ± 5.76 ^{a}	25.4 ± 1.20 ^{a}	7312.2 ± 1773.99 ^{ab}	301.9 ± 85.67 ^{a}
	Carnaval	17.2 ± 3.12 ^{a}	418.0 ± 120.76 ^{bc}	28.5 ± 9.52 ^{a}	15.8 ± 2.27 ^{b}	9306.5 ± 1646.87 ^{a}	630.0 ± 145.89 ^{a}

Table 3 (Continued)

Crop	Cultivar Name	Low *Pi*			High *Pi*		
		Root Weight (g)	**Final population (*Pf*)**	***Pf* per gram of roots**	**Root Weight (g)**	**Final population (*Pf*)**	***Pf* per gram of roots**
Phacelia	Angelia	8.7 ±1.87 [a]	137.2 ± 56.25 [c]	15.8 ± 5.71 [a]	12.3 ± 3.18 [a]	2644.6 ± 615.68 [cde]	291.4 ± 80.68 [a]
	Natra	7.9 ± 2.16 [a]	65.6 ± 28.11 [c]	12.7 ± 6.47 [a]	15.1 ± 2.53 [a]	3162.1 ±491.69 [cde]	243.9 ± 60.50 [a]
Italian ryegrass	Fedra	45.3 ± 9.35 [a]	375.6 ± 110.73 [bc]	7.9 ± 1.68 [a]	166.3 ± 37.39 [a]	921.6 ± 93.21 [de]	6.4 ± 1.06 [a]
	Meroa	57.3 ± 12.27 [a]	143.2 ± 80.86 [c]	2.8 ± 1.32 [b]	143.2 ± 14.65 [a]	1341.4 ± 121.20 [de]	9.8 ± 1.28 [a]
Rye	Dukato	58.9 ± 6.38 [a]	223.6 ± 64.93 [c]	3.9 ± 1.19 [a]	76.2 ± 19.12 [a]	2074.7 ± 224.59 [cde]	33.5 ± 6.79 [a]
	Matador	32.1 ±1.83 [b]	58.8 ± 35.14 [c]	1.8 ± 1.06 [a]	70.6 ± 9.27 [a]	1119.0 ± 309.96 [de]	15.0 ± 3.49 [b]
Summer oat	Simphony	63.9 ± 13.96 [a]	375.8 ± 108.78 [bc]	6.5 ± 2.02 [b]	60.6 ± 6.32 [a]	3956.3 ± 875.07 [bcde]	67.9 ± 13.71 [a]
	Effectiv	26.4 ± 3.15 [b]	1469.6 ± 844.99 [a]	54.4 ± 28.96 [a]	39.3 ± 4.53 [b]	6013.8 ± 1002.76 [abc]	157.7 ± 27.36 [b]
Fallow		0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 4: Reproductive factor (*Rf* ± standard error) of *Meloidogyne chitwoodi* on bird´s foot trefoil cultivars 8 weeks after inoculation with 10 J2/100 cm³ (low *Pi*) and 100 J2/100 cm³ (high *Pi*). A negative control (fallow) was subjected to the same inoculation. Different letters per column indicate significant differences between cultivars ($P \leq 0.05$).

Crop	**Cultivar Name**	**Low *Pi***		**High *Pi***	
		Rf	**Host status**	***Rf***	**Host status**
Bird's foot trefoil	Bull	1.1 ± 0.13 [a]	Maintenace host	0.9 ± 0.61	Poor host
	Barguay	0.6 ± 0.20 [ab]	Poor host	0.4 ± 0.12	Poor host
	Lotar	0.5 ± 0.17 [b]	Poor host	0.3 ± 0.06	Poor host
	Franco	0.7 ± 0.04 [b]	Poor host	0.1 ± 0.01	Non- host
Fallow		0.0 ± 0.00		0.000 ± 0.00	

Table 5: Final *Meloidogyne chitwoodi* population per gram of roots (*Pf* ± standard error) and final *M. chitwoodi* population (*Pf* ± standard error) on bird´s foot trefoil cultivars 8 weeks after inoculation 10 J2/100 cm³ (low *Pi*) or 100 J2/100 cm³ (high *Pi*). Different letters per column indicate significant differences between cultivars($P \leq 0.05$).

Crop	Cultivar Name	Low *Pi*			High *Pi*		
		Root Weight (g)	**Final population (*Pf*)**	***Pf* per gram of roots)**	**Root Weight (g)**	**Final population (*Pf*)**	***Pf* per gram of roots)**
Bird's foot trefoil	Bull	26.7 ± 4.13 ^{a}	8.9 ± 1.86	70.8 ± 26.86	11.7 ± 3.42	1800.8 ± 1215.99	200.5 ± 92.03 ^{ab}
	Barguay	5.8 ± 1.37 ^{b}	65.7 ± 51.59	103.2 ± 43.61	6.3 ± 3.06	792.4 ± 246.75	331.2 ± 110.28 ^{a}
	Lotar	16.4 ± 3.02 ^{ab}	5.8 ± 1.68	67.8 ± 26.92	15.0 ± 3.43	644.8 ± 119.97	49.2 ± 10.47 ^{b}
	Franco	18.6 ± 7.94 ^{ab}	43.5 ± 31.69	67.5 ± 26.33	7.1 ± 2.08	256.0 ± 21.84	98.9 ± 64.53 ^{ab}
Fallow		0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Table 6: Mean number (mean ± standard error) of egg masses, eggs per egg mass and percentage of plants with egg mass of *Meloidogyne chitwoodi* 8 weeks after inoculation Different letters per column indicate significant differences between plants ($P \leq 0.05$). Tomato 'Marmande' was used as a positive control.

Crop	Cultivar name	% of plants with egg mass	Egg masses/plants	Eggs/egg mass
Phacelia	Angelia	20	0.8 ± 0.5 [c]	1.52 ± 0.87 [c]
	Natra	5	0.05 ± 0.05 [c]	0.5 ± 0.50 [c]
Italian ryegrass	Meroa	90	14.3 ± 2.75 [b]	8.99 ± 2.07 [c]
	Fedra	20	0.25 ± 0.12 [c]	4.75 ± 2.40 [c]
Rye	Matador	100	11.3 ± 1.38 [b]	48.27 ± 10.99 [b]
Tomato (control)	Marmande	100	88.5 ± 3.45 [a]	107.42 ± 6.59 [a]

Table 7: The host status of different cover crops for *Meloidogyne chitwoodi* under field conditions based on the reproductive factor (*Rf* = final J2 population/initial J2 population (*Pi*)).

Cover crop	Cultivar	Date of sowing	Field growth duration (days)	Seed density	Degree days (DD_5)	*Pi* (100 cm^3 soil)$^{-1}$	*Rf*	Host status	Previous crop
Fodder radish	Terranova	23/08/2019	236	25-30 kg/ha	961,42	566	0.086	Non-host	Pea (Cher)
	Contra	19/05/2020	69	25-30 kg/ha	938,5	361	0.287	Poor host	Bird's foot trefoil (Lotar) and Italian ryegrass (Meroa)
	Doublet	10/09/2020	203	25-30 kg/ha	710,21	376	0.55	Poor host	Rye (Dukato)
	Dacapo	23/08/2019	236	25-30 kg/ha	976,57	35	6.444	Excellent host	Carrot (Salto)
		23/08/2019	236		976,57	619	0.143	Poor host	Pea (Bartesa)
		17/09/2019	203		620,47	154	0.686	Poor host	Pea (Bartesa)
		17/09/2019	203		620,47	244	0.459	Poor host	Leek (Krypton)
		19/05/2020	69		938,5	45	0.193	Poor host	Fodder radish (Terranova) and Black oat (Pratex)
		15/09/2021	153		1103,58	0	0	Non-host	Bean (Zembla)
		25/09/2021	143		583,85	115	0	Non-host	Rye (Matador)

Table 7 (Continued)

Cover crop	Cultivar	Date of sowing	Field growth duration (days)	Seed density	Degree days (DD_5)	*Pi* (100 cm^3 soil)$^{-1}$	*Rf*	Host status	Previous crop
Yellow mustard	Chacha	19/05/2020	69	10-20 kg/ha	938,5	1150	0.587	Poor host	Black salsify (Enorma) later Fallow
Italian ryegrass	Meroa	15/10/2019	175	25-40 kg/ha	374.72	19	0.321	Poor host	Bean (Zembla)
		15/10/2019	175		374.72	63	1.854	Maintenance host	Celeric (Prinz)
		6/08/2019	245		1221.8	14	42.435	Excellent host	Carrot (Solo)
		19/05/2020	114		1664.67	46	1.978	Maintenance host	Leek (Krypton) later Fallow
		28/07/2020	247		1432.79	89	0.457	Poor host	Rye (Dukato)
		8/10/2021	116		460.56	22	0.803	Poor host	Bean (Auberon)
		8/10/2021	116		460.56	12	0	Non-host	Bean (Auberon)
	Melodia	19/05/2020	114	25-40 kg/ha	1664.67	52	1.166	Maintenance host	Celeria (Prinz) later Fallow
		28/07/2020	247		1432.79	9	1.154	Maintenance host	Black e oat (Pratex) and Fodder radish (Dacapo) later fallow
Phacelia	Angelia	10/09/2020	203	7-10 kg/ha	710.21	756	1.008	Maintenance host	Rye (Matador)
		19/05/2020	69		938.5	72	3.135	Good host	Fodder radish (Dacapo) and Black oat (Pratex)
		19/05/2020	69		938.5	63	0.335	Poor host	Black oat (Pratex) and Italian ryegrass (Meroa)
		28/07/2020	247		1432.79	18	3.903	Good host	Phacelia (Angelia) and Cauliflower (Giewont)
		19/05/2020	69		1664.67	49	0.294	Poor host	Fodder radish (Dacapo) and Black oat (Pratex)
		15/09/2021	139		1103.58	5	3.675	Good host	Cauliflower (Giewont)

Table 7 (Continued)

Cover crop	Cultivar	Date of sowing	Field growth duration (days)	Seed density	Degree days (DD_5)	*Pi* (100 cm^3 soil)$^{-1}$	*Rf*	Host status	Previous crop
Black oat	Pratex	15/10/2019	183		374.72	31	1.561	Maintenance host	Bean (Ontario)
		23/08/2019	236		976.57	35	9.845	Excellent host	Carrot (Salto)
		23/08/2019	236		976.57	619	0.117	Poor host	Pea (Bartesa)
		15/10/2019	175		374.72	154	0.139	Poor host	Pea (Bartesa)
		15/10/2019	175	80-100 kg/ha	374.72	19	0.33	Poor host	Bean (Ontario)
		17/09/2019	203		620.47	30	1.889	Maintenance host	Leek (Poulton)
		15/10/2019	175		374.72	141	1.854	Maintenance host	Celeric (Prinz)
		19/05/2020	69		1002.48	48	0.169	Poor host	Fodder radish (Dacapo)
Rye	Matador	8/10/2020	175	150 kg/ha	387.42	10	12.995	Excellent host	Fodder radish (Terranova) and Black oat (Pratex)
		19/05/2020	114	80-90 kg/ha	1664.67	295	3.411	Good host	Bird's foot trefoil (Leo)
		27/07/2020	248	80-90 kg/ha	1448.5	143	1.344	Maintenance host	Phacelia (Angelia)
		28/07/2020	247	80-90 kg/ha	1432.79	22	1.154	Maintenance host	Phacelia (Angelia) and Cauliflower (Giewont)
	Dukato	8/10/2020	175	150 kg/ha	387.42	10	12.995	Excellent host	Fodder radish (Terranova) and Black oat (Pratex)
		19/05/2020	114	80-90 kg/ha	1664.67	96	5.205	Excellent host	Bird's foot trefoil (Lotar)

LEGENDS FOR FIGURES

Fig. 1: The mean final populations (*Pf*) of *M. chitwoodi* recovered from a combination of soil and root fractions from cover crops 8 weeks after inoculation with an initial nematode density of 10 J2/100 cm^3 (low *Pi*). Significant differences ($P \leq 0.05$) between cultivars of the same cover crop are indicated with different letters. Error bar indicates standard error of final population (*Pf*). *Pi* = total number of *M. chitwoodi* inoculated.

Fig. 2: The mean final populations (*Pf*) of *M. chitwoodi* recovered from a combination of soil and root fractions from cover crops 8 weeks after inoculation with initial nematode density of 100 J2/100cm^3 (high *Pi*). Significant differences ($P \leq 0.05$) between cultivars of the same cover crop are indicated with different letters. Error bar indicates standard error of final population (*Pf*). *Pi* = total number of *M. chitwoodi* inoculated.

Fig. 3: *Meloidogyne chitwoodi* in the roots of different cover crops monitored 2, 7, and 14 days post inoculation (dpi). Plants were inoculated with approximately 200 freshly hatched J2's. Tomato 'Marmande' was used as a positive control. Bars represent the mean and standard error of the mean. Letters on bars indicate statistic significant differences ($P \leq 0.05$) for each monitored time point respectively.