Ringtest to evaluate four methods of resistance testing in Fodder Radish against *Meloidogyne chitwoodi*

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1 Introduction

The root-knot nematode *Meloidogyne chitwoodi* is a quarantine nematode in the EU. Especially in the regions with sandy soils this nematode is a major pest and can cause severe damage to economically important crops like potato, black salsify, carrot, peas and sugar beet. Due to it's quarantine status propagation material (such as seed potatoes, bulbs and strawberry) needs to be free of this nematode species.

1.1 *Meloidogyne chitwoodi*

Root knot nematodes, like *M. chitwoodi*, are endo-parasitic. The nematodes penetrate the roots of the host plant and move intercellularily. In spring, when soil temperatures rise above 5 °C, mature Meloidogyne eggs hatch spontaneously. These infectious juveniles (J2) penetrate the roots of host plants and start to feed. The J2 is together with the adult male the only mobile stage. Female nematodes complete their lifecycle inside the roots and produce new eggs. *M. chitwoodi* multiplies rapidly at warm temperatures, completing two or more generations in long, warm growing seasons under Dutch conditions. Therefore a population density of 1 per 100 ml soil at planting could increase to several thousands per 100 ml at harvest. *M. chitwoodi* normally causes minimal cell damage and therefore no huge reduction in total yield, compared to for example cyst nematodes. However, *M. chitwoodi* may cause severe quality damage, which reduces the economic value and often lead to unmarketable products (pictures 1 and 2).

Picture 1. Damage on tap roots of carrot caused by *M. chitwoodi*

Picture 2. Galling of potato tubers caused by *M. chitwoodi*

1.2 Nematode Control Strategies

Since chemical control measures become more and more restricted, there is an urgent need to develop other methods to control nematodes.
A well chosen crop rotation, appropriate to the local nematode situation can be an important measure to control nematode damage. Knowledge of host suitability and damage thresholds of crops for nematodes are indispensable to design a crop rotation that will diminish or prevent nematode damage. Also the choice of green manure crops is of significant importance within a sound crop rotation, because the multiplication of nematodes on green manure crops can be considerable. Most green manure crops are good hosts to *M. chitwoodi*. Most common fodder radish (*Raphanus* sativus) cultivars are a moderate host to *M. chitwoodi*. Recently different breeding companies started to select and breed on fodder radish cultivars with high levels of resistance against *M. chitwoodi*.

1.3 Ring test project

To measure levels of resistance in fodder radish cultivars a reliable, objective and cost effective testing method is required. In 2006 German and Dutch plant breeder's organizations (Bundesverband Deutscher Pflanzenzüchter; BDP and Plantum), a number of research institutes (PRI, PPO (WUR) and JKI) and breeding companies started this "Ring test project".

Aim of the project is to compare different methods to quantify the level of resistance in fodder radish varieties against *Meloidogyne chitwoodi*. The final aim is to find the best method and criteria which can serve as an European standard for evaluation of the level of resistance in fodder radish (*Raphanus sativus*) against *M. chitwoodi*.

In total four fodder radish genotypes, delivered by various breeding companies, and one reference (susceptible) fodder radish cultivar were investigated with three different lab tests and one field-experiment. The three different lab tests, performed with the same *M. chitwoodi* population and fodder radish genotypes as the field experiment, were conducted by Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA, J. Hallman), HZPC (D. Boomsma & L. Altena) and Plant Research International (PRI; H. Kok & L. Poleij). The field experiment was carried out by PPO (G. Korthals, J. Visser).

Breeders which participated in this project were:
Joordens (E. Wilken), Limagrain (J. Velthuis), Freudenberger (J. Bestajovsky), Eurograss (P. Lammers), Vandijke Semo (H. van Dijke/M. Hellendoorn) and PHPetersen (M. Schlathoelter).

The whole project was coordinated by PPO agv.
The project was financed by the different breeding companies, Biologische Bundesanstalt für Land- und Forstwirtschaft , Productschap Akkerbouw (PA, Product board of Arable products) and the Dutch government (LNV).

2 Statistical analysis

All data were statistical analyzed using Genstat Windows 10th edition. Data of nematode sampling obtained by the field experiment were ^{10}log transformed to stabilize the variance and analyzed with ANOVA to asses the effect of the treatments on the *M. chitwoodi* population. The means obtained after ^{10}log transformation are back transformed. These back transformed means (called medians) are less influenced by extremes.
Due to natural variation within the experimental field small differences were found in population densities of *M chitwoodi* (Pi) before sowing of the fodder radish genotypes. To compensate for these small differences, statistical analysis of the nematode counts was preformed with Pi as co-variable.
Results of nematode sampling of two plots, out of a total of 28 plots, were very divergent (statistical outliers). So all data obtained from these plots were removed from the statistical analysis
The five fodder radish genotypes, delivered by the breeding companies, were coded by the statistician of PPO agv (W. van den Berg) and distributed to the different laboratories and the field station of PPO agv in Vredepeel (responsible for the field experiment).

The referent fodder radish variety included in all experiment is coded as **FR-C**. On request of the participant (ring test meeting on the 9th of July 2008, Vredepeel) this referent is decoded.

3 Laboratory and greenhouse experiments

Three different lab / greenhouse tests were performed (BBA, PRI and HZPC).

Nematode suspension of *M. chitwoodi* (Smakt population) required for these lab and greenhouse experiments was prepared by PRI.
Before distribution the purity of the nematode suspension was tested by molecular techniques and microscopically.

3.1 Greenhouse experiment BBA

Johannes Hallman of the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) carried out a greenhouse test. In the first test the multiplication rate was low probably due to unfavourable climate conditions (high temperatures). To obtain more reliable results the test was carried out a second time.

3.1.1 Materials and methods

In the first experiment (**BBA-1**) 120 seedlings per fodder radish genotype were tested. Fodder radish cv. Siletina was added to the experiment as an internal control, to determine developmental stage of egg masses. Seeds were sown in transparent plastic containers (40 x 20 x 120 mm) filled with silica sand. Two seeds per container were sown and containers were placed in a greenhouse. All 120 containers of one genotype were placed in one box made of stainless steel; the bottom of the box was covered with fleece. Seven days after sowing the seedlings were thinned to obtain one plant per container and inoculated with approx. 500 juveniles in 2 ml tap water. Inoculum density was counted in 1 ml before, during and at the end of inoculation; respectively 515, 633, 415 juveniles. Inoculation was performed using a dispensette, adding an average of 521 juveniles per plant.
Plants were watered regularly and fertilized once a week with Steiner-I solution. Temperature was recorded with a thermograph; temperature minimum was 14°C, temperature maximum was 38°C.
Fifty- eight days after inoculation half of the newly produced eggs (on Siletina) contained juveniles and the test was terminated following two approaches:

A) 60 seedlings were used to determine the number of egg masses
B) 60 seedlings were used to determine the number of hatched juveniles

Stems were cut off and discarded and containers were then soaked in tap water. Roots were carefully washed free of sand particles and:

A) stained in a 0.2% solution of red food stain for better detection of egg masses and number of egg masses per root system was counted
B) roots were placed on a sieve (D = 6 cm) sitting in glass Petri plate (D = 13 cm) and incubated in a misting chamber; hatched juveniles were collected weekly for 4 weeks.

The number of egg masses seemed to be very low, therefore the experiment was replicated.

In the second experiment (**BBA-2**) 120 seedlings per fodder radish genotype were tested and tomato cv. Moneymaker, known as a good host for *M. chitwoodi*, and fodder radish cv. Siletina were added to the experiment as references. Seeds were planted in the same way as in experiment 1, and containers were placed in a growth chamber at 20°C during 12 hrs light period and 15°C for 12 hrs at dark. Seven days after sowing seedlings were then inoculated with approx. 414 juveniles + 198 eggs in 1.5 ml tap water. It was assumed that 50% of the eggs will produce an infective juvenile, i. e. total number of inoculum would be 513 juveniles/plant. Inoculum density was recorded in 1 ml before, during and at the end of inoculation; respectively 292 L + 146 E, 307 L + 152 E, 269 L + 108 E.; average = 289 L + 135 E. Inoculation was performed using a dispensette, adding an average of 535 juveniles (**(**289 L + 50% of 135**)** x 1,5 ml) per plant.

Sixty-three days after inoculation half of the newly produced eggs (on Siletina) contained juveniles and the test was terminated; stems were cut off and discarded and containers with plants were then soaked in tap water. Roots were carefully washed free of sand particles and stained in a 0.4% solution of red food stain for better detection of egg masses; number of egg masses per root system was counted as described above.

3.1.2 Results and discussion

The results of the first greenhouse test of BBA are listed in table 1.

No reference was included in this experiment. Overall nematode infestation in this experiment seemed very low. The low infestation resulted in an overall high variability within each treatment. The reasons for the low infestation can be manifold but are most likely due to unfavorable temperature conditions during the test period in summer (resistance testing, by BBA, is otherwise done in early spring at cooler temperatures).
Within the limitations of the overall low nematode infestation, the number of egg masses (method A) was highest for fodder radish (FR) C with 1.67 egg masses/plant. On 37 percentage of the plants of FR C egg masses were counted. Only very few egg masses were found on FR A, B and E, and number of infested plants of these genotypes was also very low. No egg masses were detected in FR D.
The low infestation also resulted in a low number of hatched juveniles (method B) and an overall high variability within each treatment. The results of method B are not presented in this report because of the high variability and the fact that the results are comparable to the results obtained by method A (number of egg masses per plant).

Table 1. Results of the first experiment of BBA; number of infested plants and egg masses per plant.

Objects	Number of plants observed	***Infested plants*** number of plants	percentages of plants	***Egg masses*** maximum number/plant	average number/plant (SD *)	
Fodder radish **A**	57	3	5%	2	0,07	(0,32)
Fodder radish **B**	56	1	2%	1	0,02	(0,13)
Fodder radish **C**	54	20	37%	12	1,67	(2,93)
Fodder radish **D**	49	0	0%	0	0,00	(0,00)
Fodder radish **E**	53	2	4%	5	0,11	(0,70)

* Standard Deviation

Nematode infestation in the first experiment seemed very low, and therefore the experiment was replicated. The results of the second experiment of BBA are shown in table 2.
The multiplication of *M. chitwoodi* on the references tomato cv. Moneymaker and FR cv. Siletina was good. On all tomato plants egg masses were counted, and for Siletina 62 % of the plants were infested. Average number of egg masses per plant counted on tomato and cv. Siletina were respectively 54 and 18.
For the tested FR genotypes, the number of infested plants and number of egg masses per plant were highest for FR C with 55 % of the plants infested and 17 egg masses/plant. Only very few infested plants and egg masses (between 0,05 and 0,18 per plant) were found in FR A, B and D. No egg masses were detected on FR E. This is in contrast to the results of the first experiment, where FR D had no egg masses and FR E a few.

Table 2. Results of the second experiment of BBA; number of infested plants and egg masses per plant.

Objects	Number of plants observed	***Infested plants*** number of plants	***Infested plants*** percentages of plants	***Egg masses*** maximum number/plant	***Egg masses*** average number/plant (SD *)
Tomato cv. Moneymaker	34	34	100	96	54,15 (19,51)
Fodder Radish cv. Siletina	55	34	62	89	18,10 (26,80)
Fodder radish **A**	120	4	3	2	0,05 (0,29)
Fodder radish **B**	120	3	3	17	0,17 (1,56)
Fodder radish **C**	118	65	55	103	16,99 (22,94)
Fodder radish **D**	118	6	5	7	0,18 (0,91)
Fodder radish **E**	119	0	0	0	0,00 (0,00)

* Standard Deviation

3.1.3 Costs of the experiment

Based on experiment BBA-I, with five fodder radish genotypes and one reference with **60** replications each the following time was spend:
1) experimental set-up: 1 day => 8 h
2) watering, fertilization, etc; 10 min/day over 64 days => 10 h 40 min
3) extraction of root systems and staining of egg masses => 20 h
4) counting of stained egg masses => 20 h
5) data analysis and reporting => 8 h

In total 66 h 40 min were spent to carry out the experiment. The costs of this experiment (without costs of material, inoculum and green house), and charging a (fictive) rate of €80,- per hour will be €5333,-. Divided by five fodder radish genotypes and the control (= 6 treatments) the costs per genotype are **€885,-**.

3.2 Greenhouse experiment PRI

Hans Kok and Leo Polij of Plant Research International (PRI) in Wageningen carried out a greenhouse test.

3.2.1 Materials and methods

60 plants per fodder radish genotype were tested. Seeds were planted in 400 ml clay pots filled with silver sand; one seed per pot. Pots were placed in a temperature controlled greenhouse; 20°C during 12 hrs light period. Pots were arranged in five blocks, with six plants of each genotype within each plot. One pot with tomato cv. Moneymaker, known as a good host for *M. chitwoodi*, was added to each plot as a reference. Plants were watered regularly and fertilized once a week with a liquid fertilizer.
Each plant was inoculated four weeks after sowing with approx. 550 juveniles by adding 4 ml of a *M. chitwoodi* suspension (136 J2/ml) to the soil with a micropipette.
Eight weeks after inoculation the soil was washed away from the roots, and fresh weight of each root system was determined. Roots were stained in a 0,02% (w/v) solution of Phloxine B for better detection of egg masses and number of egg masses per root system was counted.

3.2.2 Results and discussion

In table 3 the results of the greenhouse test of PRI are presented.
The multiplication of *M. chitwoodi* on the reference tomato cv. Moneymaker was good. All plants showed egg masses and the average number of egg masses per plant was high (190).
For the tested FR genotypes, the number of infested plants and number of egg masses per plant were highest for FR C with 57 % of the plants infested and an average of 9 egg masses/plant. Only very few infested plants and egg masses were found on FR A and E. No egg masses were detected on FR B and D.

Table 3. Results of the greenhouse experiment of PRI; number of infested plants and egg masses per plant.

Objects	Number of plants observed	***Infested plants*** number of plants	percentages of plants	***Egg masses*** maximum number/plant	average number/plant (SD *)	
Tomato cv. Moneymaker	5	5	100%	321	190	(93)
Fodder radish **A**	30	2	7%	3	0,17	(0,65)
Fodder radish **B**	30	0	0%	0	0,00	(0,00)
Fodder radish **C**	30	17	57%	130	9,20	(24,50)
Fodder radish **D**	30	0	0%	0	0,00	(0,00)
Fodder radish **E**	30	4	13%	3	0,27	(0,74)

* Standard Deviation

3.2.3 Costs of the experiment

To carry out the experiment with five fodder radish genotypes and one reference with **30** replications 80 hours were spent.
The costs of this experiment (without costs of material, inoculum and green house), and charging a (fictive) rate of €80,- per hour will be €6400,-. Divided by five fodder radish genotypes and the control (= 6 treatments) the costs per genotype are **€1067,-**.

3.3 Laboratory experiment HZPC

An in vitro test was performed by D. Boomsma and L. Altena of HZPC Holland BV, Department Research in Metselawier.

3.3.1 Materials and methods

For the in vitro test a "clean" (sterile) inoculum is required. Therefore the inoculum distributed by PRI was multiplied on a so called "clean culture" (HZPC protocol). After eight weeks of incubation this culture was rinsed. The juveniles were hatched and sterilized as described by the HZR sterilisation procedure. The effectiveness of the sterilisation was checked by a bouillon-test. After two weeks the inoculum was found to be "clean" and could be used for the in vitro test.
Per fodder radish genotype 120 seedlings were tested and tomato cv. Moneymaker (27 plants) and potato cv. Desiree (18 plants), both known as good hosts for *M. chitwoodi*, were added to the in vitro experiment as references.
Of each of the five fodder radish genotypes 180 seeds were sterilized and sown on Petri dishes filled with HZR culturing medium. Approximately 18 days after sowing, 120 plants per fodder radish genotype and the two references were inoculated with 400 juveniles.
Eight weeks after inoculation the number of egg masses per root system (plant) was counted.

3.3.2 Results and discussion

Table 4 shows the results of the laboratory experiment of HZPC.
Multiplication of *M. chitwoodi* on the references tomato cv. Moneymaker and potato cv. Desiree was good. On all tested tomato and potato plants egg masses were counted. An average of 16 and 47 egg masses per plant were respectively found on tomato and potato.
For the tested FR genotypes, the number of infested plants and number of egg masses per plant were highest for FR C with 72 % of the plants infested and an average of 9 egg masses/plant. Only very few infested plants and egg masses were found on FR A, B and E. No egg masses were detected on FR D.

Table 4. Results of the laboratory experiment of HZPC; number of infested plants and egg masses per plant.

Objects	Number of plants observed	***Infested plants*** number of plants	***Infested plants*** percentages of plants	***Egg masses*** maximum number/plant	***Egg masses*** average number/plant (SD *)
Tomato cv. Moneymaker	20	20	100%	30	16 (7,1)
Potato cv. Desiree	17	17	100%	64	47 (12,1)
Fodder radish **A**	120	1	1%	26	0,22 (2,37)
Fodder radish **B**	120	5	4%	28	0,59 (3,39)
Fodder radish **C**	119	86	72%	77	9,39 (13,51)
Fodder radish **D**	120	0	0%	0	0,00 (0,00)
Fodder radish **E**	120	3	3%	17	0,23 (1,70)

* Standard Deviation

3.3.3 Costs of the experiment

To carry out the experiment with five fodder radish genotypes and two references with **120** replications 40 hours were spent.
The costs of this experiment (without costs of material, inoculum and green house), and charging a (fictive) rate of €80,- per hour will be €3200,-. Divided by five fodder radish genotypes and the control (= 6 treatments) the costs per genotype are **€533,-**

3.4 Comparing lab and greenhouse tests

An overview of the results of the different lab / greenhouse tests is presented in figure 1 and 2.
Figure 1 shows the percentages of infested plants determined by the three different testing methods.
In all tests the highest percentage of infested plants and mean number of egg masses per plant was recorded on **FR C**. The highest percentage infested plants of FR C was reported by HZPC (72%) the lowest by the first experiment of BBA (37%). In all tests no or only low numbers of infested plants were recorded for FR A, B, D and E.
For **FR A** percentage of infested plants ranged from 1% (HZPC) up to 7% (PRI) and in all tests infested plants were observed. PRI did not record infested plants for **FR B**. Respectively 4, 2 and 3% infested plants of FR B were found by HZPC and BBA (first and second experiment). Only in the second experiment of BBA egg masses were counted on **FR D**. In all other experiment no infested plants of FR D were observed. In all experiments, except the second experiment of BBA, infested plants of **FR E** were observed. The highest percentage infested plants of FR E was reported by PRI (13%) the lowest by HZPC (3%).

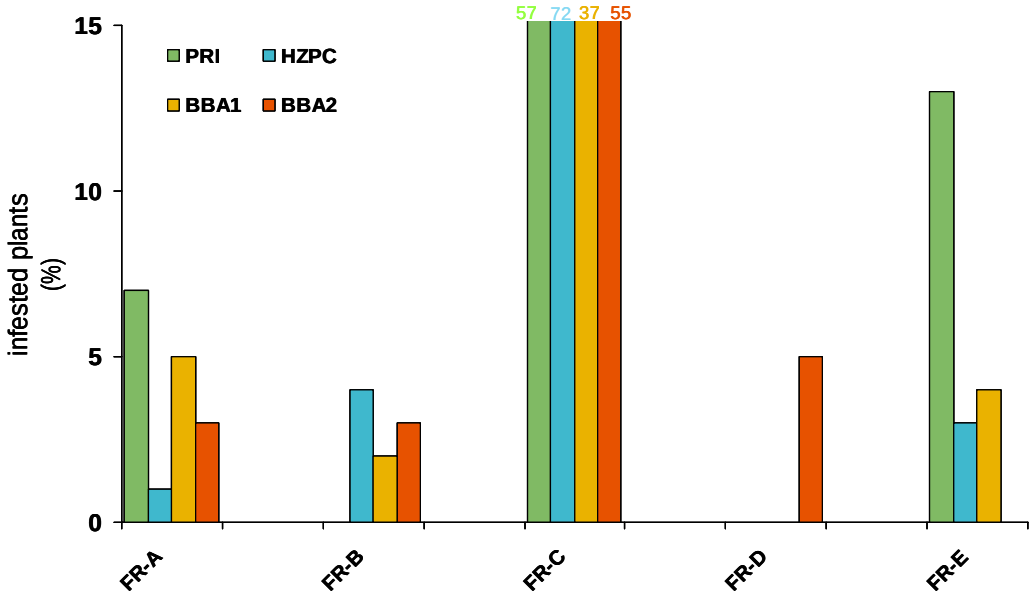

Figure 1. Percentage of *M. chitwoodi* infested plant of five different fodder radish genotypes determined by four different tests.

The average number of egg masses per plant of the different fodder radish genotypes estimated by the four experiments are shown in figure 2.
The average number of egg masses per plant and the percentages of infested plants of the five fodder radish genotypes, estimated by the different testing methods, seems rather similar. In all tests the highest mean number of egg masses per plant was recorded on **FR C**, ranging from 1,67 (BBA 1) up to 17 (BBA 2). In all experiment FR A, B, D and E had no or only low numbers of egg masses per plant, and differences between experiments are relatively small. However PRI did not count any egg masses on FR B and in the second experiment of BBA egg masses were found on FR D and not on FR E. Which is in contradiction to the results of the other three experiments.

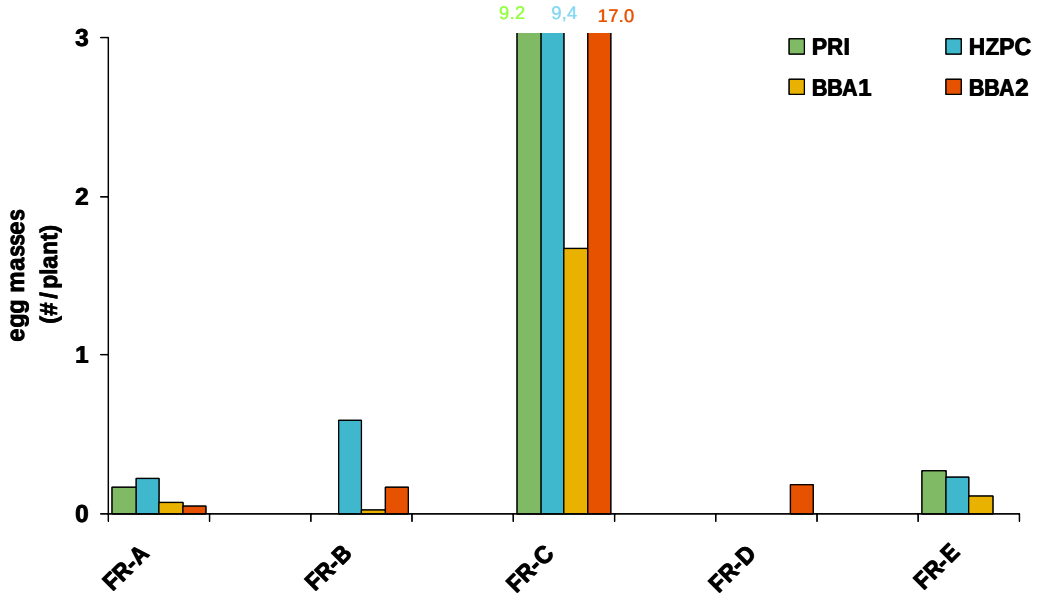

Figure 2. Average number of egg masses of *M. chitwoodi* per plant of five different fodder radish genotypes determined by four different tests

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Frequency (%) histograms of plants classified to number of egg masses per plant determined by the different testing methods are shown in appendix 1.

4 Field experiment

The results of the different lab and greenhouse tests will be compared to the level of host suitability of the fodder radish genotypes estimated by a field experiment, and to tuber quality of the proceeding potato crop (grown after fodder radish).

4.1 Materials and methods

The field experiment was carried out on a sandy soil at Smakt (Limburg,) that is naturally infested with *Meloidogyne chitwoodi*.

4.1.1 Experimental design

The experimental field was divided into twenty-eight plots of 6 x 6 m each. The plots were arranged in four blocks, in which each of the objects (five fodder radish cultivars, Italian ryegrass and fallow) was represented once and distributed randomly. The host suitability of the five fodder radish cultivars to *M. chitwoodi* is determined by comparing nematode population densities before sowing (May 2006) and after the growing season (Oct. 2006). The *M. chitwoodi* population after growing the five fodder radish cultivars is compared to *M. chitwoodi* populations after growing Italian ryegrass (good host) and fallow. Fallow is included to determine the natural decline of the nematode population in absence of a host plant.
In 2007 potatoes cv Asterix were grown on all the plots to determine the effect of the objects on a susceptible proceeding crop. This method proved to be very sensitive to investigate the consequences of infestations with low numbers of *M. chitwoodi*, which are to be expected after growing resistant fodder radish cultivars.

4.1.2 Field experiment

Host suitability test (2006)
In April 2006 the field was ploughed and a soil sample was taken for analysis on macro elements N (nitrogen), P (phosphate) and K (potassium) as well as organic matter content, pH and % silt content.
In May, after soil sampling for analysis on nematode population densities, the different fodder radish genotypes and the Italian ryegrass were sown. During the whole growing season four plots were kept fallow by herbicide application monthly. Crop development was observed regularly. The fodder radish crops were mown at a height of 40 cm above ground level, to prevent seeding and stimulate vegetative growth.

In September the green manure crops were mown, crop debris removed from the plots and the stubble incorporated into the soil. After two weeks soil samples were taken to determine the final population densities.
The most important details of the trail are listed in table 6.

Growing a susceptible cash crop (potato) in 2007
In April 2007 the experimental field was ploughed and in May potatoes (*Solanum tuberosum* cv Asterix) were planted at 30-cm spacing on 75-cm rows (8 rows per plot). All cultural practices like fertilization and crop protection against diseases were in line with standard commercial potato production in the Netherlands.
Crop development was observed regularly. Data of emergence and percentages of soil covered by green foliage were determined.
In October the potatoes were harvested mechanically. Crop yield was established by gross yield from net plots (4 rows of 4,5 m length). Tubers were sorted by size(< 30mm, 30 – 50 mm and > 50 mm) and weights per size category was determined. A representative sample of 5 kg was taken from these tubers to establish underwater weight, which is an indication of the starch amount in the tuber.

To determine the severity of root-knot nematode infection of the potato tubers out of each net plot 2 x 30 tubers were selected randomly and assessed for tuber symptoms. Tubers were categorized, based on severity of infection by *M. chitwoodi* (table 5). Criteria for this classification are listed in the table below.

Table 5. Classification of potato tubers based on symptoms of *M. chitwoodi* infection.

Category	Symptoms, galls	Eggs, visible after peeling
0	No galls	No
1	No galls	Yes
2	< 30% of tuber galled	Yes
3	30 – 100% of tuber galled	Yes
4	Tuber extremely galled	Yes

Picture 3. External and internal symptoms on potato tubers caused by infection of *Meloidogyne chitwoodi*.

For each sample of 30 tubers the number of tubers per category was determined. Out of these data the **T**uber **G**alling-**I**ndex (TGI) was calculated by the formula below.

$$\text{TG-index} = \frac{((\#\text{ tubers cat. } 0 + 1) \times 0) + (\#\text{ tubers cat. } 2 \times 10) + (\#\text{ tubers cat. } 3 \times 33) + (\#\text{ tubers cat. } 4 \times 100)}{\text{Number of tubers observed}}$$

The severity of tuber infection per plot is expressed by the average of the TGI of the two tuber samples per plot.

Table 6. Experimental details, field trial for host suitability test of fodder radish cultivar to *M. chitwoodi*, Smakt 2006-2007.

Location	:	Smakt (The Netherlands)
Soil	:	Sandy soil
Silt content	:	< 1,5%
PH-KCl	:	5,6
% organic matter	:	3,0
Pre-crop (2005)	:	Carrot
Fodder radish (*Raphanus sativus*)		
cultivars	:	Code A to E
Sowing density	:	30 kg/ha
Sowing date	:	30 May 2006
Italian ryegrass (*Lolium multiflorum*)	:	
cultivar	:	Bartali
Sowing density	:	30 kg/ha
Sowing date	:	30 May 2006
Potato		
cultivar	:	Asterix
seed spacing	:	30 cm
Row spacing	:	75 cm
Planting date	:	26 April 2007
Harvesting date	:	23 October 2007
Gross plot	:	6 x 6 m
Net plot nematode sampling	:	2 x 2 m
Net plot potato yield	:	3 x 4,5 m
Pi sampling	:	30 May 2006
Pf sampling, after growing fodder radish	:	30 October 2006
Pf sampling, after growing potato	:	23 October 2007

4.1.3 Nematode sampling

Soil samples for analysis on nematode population densities were collected in each net plot on three dates. Before sowing fodder radish (Pi, May 2006), after growing fodder radish (Pf-1, October 2006) and after growing the test crop potato (Pf-2, Oct. 2007).

Each soil sample (1,5L) was based on 40 cores (diameter 13 mm) taken in a regular pattern within the net of each plot from the top 25 cm of the soil. Nematodes were extracted from a 100 ml sub-sample by using an Oostenbrink elutriator. The remaining root-organic matter fraction was incubated at 20°C for 28 days to allow egg hatch.

4.2 Results and discussion

The five fodder radish cultivars and Italian ryegrass were sown in May 2006. Germination and crop development were good, and no remarkable differences between fodder radish genotypes were observed. To prevent seeding the fodder radish crops were mowed twice (25 July and 18 September) approximate 40 cm above ground level. Soil samples, taken before (May) and after (October) growing the green manure crops, were analyzed and species and infestation levels of the nematode population were determined. Apart from *M. chitwoodi* no, or only low numbers of other plant parasitic nematodes were found.

Initial population densities of *M. chitwoodi*

In spring 2006 the experimental field was moderately to heavily infested with *M. chitwoodi*. Before growing the green manure crops the average population density of *M. chitwoodi* was 255 larvae (L) per 100 ml soil (table 7). The average population density per object ranged from 146 L/100 ml soil for Italian rye grass to 488 for fodder radish E. The average population density of all the fodder radish objects did not significantly differ from the average population density of black fallow and, except for fodder radish E, not from Italian ryegrass. The average *M. chitwoodi* infestation of the Italian ryegrass plots was significantly lower compared to the average density of the fodder radish E plots (table 1).

Final population densities of *M. chitwoodi*

After the growing season (October) soil samples were take to determine the effect of the crops on the *M. chitwoodi* population (final population; Pf. Table 7).

The effects of the references black fallow and Italian ryegrass were conform expectations. The *M. chitwoodi* population decreased strongly after black fallow. The infestation declined to an average of 1 L/100 ml soil. After growing Italian ryegrass, known as a good host for *M. chitwoodi*, the population increased very strong to an average density of 6755 L/100 ml soil.

All fodder radish genotypes, except fodder radish C, decreased the *M. chitwoodi* population to very low numbers. Strongest decline was realized by fodder radish D, and final population density was comparable to population density after black fallow. Although fodder radish genotypes A, B and E also decreased the *M. chitwoodi* population very strong, final population densities were significantly higher compared to black fallow. After growing fodder radish C the *M. chitwoodi* population increased, but not as strong as after growing Italian ryegrass.

A crop or cultivar will be classified as non-host if decrease of the nematode population is (statistically) equal to the natural decline of the population after black fallow. Based on the results of the field experiment only FR D can be classified as non-host for *M. chitwoodi.* FR A, B and E can be classified as poor hosts and FR C as moderate/good host for *M. chitwoodi.*

Table 7. *Meloidogyne chitwoodi* population densities before (Pi, May 2006) and after (Pf-1, Oct 2006) growing the green manure crops, Smakt 2006.

	Pi (L/100 ml soil)			Pf (L/100 ml soil)		
Objects	10 LOG	median		10 LOG	median	
Black fallow	2.29	194	a b	0.23	1	a . . . .
Italian ryegrass	2.17	146	a .	3.76	6755	. . . . e
Fodder radish **A**	2.47	296	a b	0.90	7	. b c . .
Fodder radish **B**	2.38	239	a b	0.86	6	. b c . .
Fodder radish **C**	2.33	212	a b	2.60	372	. . . d .
Fodder radish **D**	2.52	332	a b	0.53	2	a b . . .
Fodder radish **E**	2.69	488	. b	1.09	13	. . c . .
F prob	*0.253*			*< 0,001*		
lsd	*0.431*			*0.477*		

Objects within a column followed by the same letter do not statistically differ

Effects on proceeding potato crop

In 2007 potato cv. Asterix, known as a susceptible cultivar, was grown on all plots.
Potatoes were planted on April 26. No significant differences in emergence and crop development between objects were observed during the whole growing season. Foliage of potatoes grown on Italian ryegrass and black fallow plots started to show symptoms of die back/ageing first, but differences to other plots were small and not significant.

Tubers were mechanically harvested on 23 October and sorted. Lowest potato yield was obtained after Italian ryegrass and FR C, but only Italian ryegrass decreased yield significantly compared to black fallow and all other FR genotypes (table 8).
UnderWater Weight (UWW) of potatoes grown after Italian ryegrass and FR C was significantly lower than the UWW of potatoes grown after black fallow. FR A, B, D and E had no effect on the UWW of the potatoes, compared to black fallow.

Tubers were evaluated for root-knot nematode infection and Tuber Galling -index (TG-index) per object is calculated. No or only a few galls are visible when TG-index is < 10. When TG-index is between 10 – 20, tubers are slightly galled and quality and economic value are reduced. Tubers are heavily galled when TG-index rises above 20. The quality of these tubers will not suffice quality standards used by potato processors and therefore may lead to an unmarketable product.

Tuber quality of potatoes grown after black fallow was relatively good (TG-index = 5,2). Italian ryegrass, a good host for *M. chitwoodi* as pre-crop resulted in moderate to heavily galled potato tubers.
FR C decreased tuber quality also. Tubers were moderately galled, and TG-index did not significantly differ from TG-index of the Italian ryegrass object.
Compared to Italian ryegrass and FR C all other FR genotypes had a positive effect on tuber quality.
TG-index was lowest for FR D (4,7) followed by FR E, FR A and FR B. Tuber quality was relatively good for these FR genotypes and statistically equal to the tuber quality observed after black fallow.

After potato harvest soil samples were taken in each plot to determine final population levels (Pf-2) of *M. chitwoodi*. Potato did increase population levels in all plots. Highest population densities of *M. chitwoodi* were observed after Italian ryegrass and FR C. Lowest population density was recorded after FR D, but was not significantly different to densities after black fallow, FR A, FR B and FR E. The *M. chitwoodi* population density after FR D is significantly lower compared to Italian ryegrass and FR C. All other fodder radish genotypes did not statistically differ from Italian ryegrass and FR C.

Table 8. Yield and tuber quality (galling) of potato grown after different green manure crop, and *Meloidogyne chitwoodi* population densities after harvest (Pf-2, October), Smakt 2007.

	Potato yield	*Tuber quality*	Pf – after potato (L/100 ml soil)	
Objects	(ton/ha)	(TG-index)	10 LOG	median
Black fallow	67,7 . b	5,2 a .	2,62	418 a b
Italian ryegrass	54,8 a .	19,6 . b	3,10	1258 . b
Fodder radish **A**	70,6 . b	5,8 a .	2,79	617 a b
Fodder radish **B**	70,6 . b	7,1 a .	2,90	783 a b
Fodder radish **C**	63,5 . b	15,7 . b	3,03	1070 . b
Fodder radish **D**	67,2 . b	4,7 a .	2,52	333 a .
Fodder radish **E**	67,0 . b	5,3 a .	2,66	462 a b

Object within a column followed by the same letter do not statistically differ

The *M. chitwoodi* population densities determined after growing the green manure crop (Pf, October 2006) is highly correlated with the quality of the tuber of the potatoes grown the year after (figure 3).
Lower numbers of *M. chitwoodi* resulted in a better quality of the potato tubers.

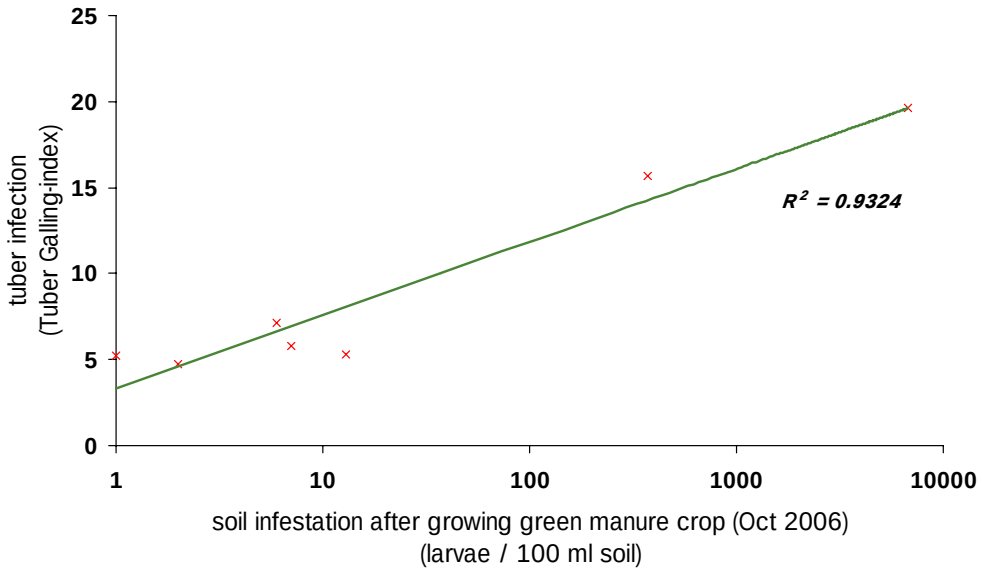

Figure 3. Relation between initial population densities of *M. chitwoodi*, established after green manure crops, and tuber quality of proceeding potato crop.

5 Comparing field experiment to lab and greenhouse experiments

The results of the lab / greenhouse experiments were compared to the results of the fodder radish genotypes obtained by the field experiment; *M. chitwoodi* population after growing fodder radish (Pf) and after proceeding potato crop (Pf-2) and tuber quality of the potatoes. Correlations between the different parameters are listed in table 9.
As already mentioned (chapter 3.4) results of the different lab / greenhouse tests are highly comparable. Correlations (r^2) calculated between tests and parameters are very high and range from 0,912 up to 1 (0,9996).
Results of the different lab and greenhouse tests are also highly related to results (Pf and tuber quality) of the field experiment. Correlations are very high and range from 0,8870 up to 0,9998.
Results on *M. chitwoodi* population after growing fodder radish (Pf) correlated best to average number of egg masses per plant. Highest correlation between these parameters was obtained by the experiment of PRI. Tuber quality (TG-index) was correlated best to the average number of egg masses per plant determined by HZPC.

Table 9. Correlation matrix, comparing results of laboratory-, greenhouse – and field experiments to determine levels of resistance of fodder radish genotypes for *Meloidogyne chitwoodi*.

	% infested plants PRI	% infested plants HZPC	% infested plants BBA 1	% infested plants BBA 2	egg masses per plant PRI	egg masses per plant HZPC	egg masses per plant BBA 1	egg masses per plant BBA 2	Tuber quality (TG-index)	Pf (after FR)	Pf-2 (after potato)
% infested plants PRI	1.000										
% infested plants HZPC	0.944	1.000									
% infested plants BBA 1	0.972	0.981	1.000								
% infested plants BBA 2	0.912	0.986	0.967	1.000							
egg masses per plant PRI	0.958	0.996	0.988	0.990	1.000						
egg masses per plant HZPC	0.940	1.000	0.983	0.988	0.996	1.000					
egg masses per plant BBA 1	0.969	0.995	0.992	0.983	0.999	0.994	1.000				
egg masses per plant BBA 2	0.942	0.997	0.981	0.995	0.999	0.997	0.995	1.000			
Tuber quality (TG-index)	0.887	0.976	0.953	0.948	0.958	0.978	0.956	0.962	1.000		
Pf (after FR)	0.956	0.998	0.987	0.990	1.000	0.997	0.999	0.999	0.961	1.000	
Pf-2 (after potato)	0.581	0.691	0.686	0.634	0.651	0.700	0.653	0.656	0.822	0.657	1.000

Although correlations are high some differences between lab / greenhouse tests and the results of the field experiment were observed. PRI did not count any galls on FR B in contradiction to the results of the other tests. Based on the results of the field experiment FR B would be classified as poor host (Pf of FR B is significant higher compared to Pf of black fallow).
In the second experiment of BBA egg masses were counted on FR D and not on FR E. The other lab / greenhouse tests showed opposite results; no egg masses on FR D and few egg masses on FR E. The results of the field experiment indicated that FR D would be a non host for *M. chitwoodi* (Pf of FR D is significantly equal to Pf of black fallow).

6 Conclusions

1. Results of the different lab /green house tests are highly correlated

2. The correlations between lab /green house tests and results of the field experiment are very high

3. Lab / green house tests have best correlations with final population densities (Pf-after fodder radish) of the field experiment, very good correlations with tuber quality (TG-index) and much less correlations with population densities after growing potato (Pf-2).

4. Egg masses per plant is the most sensitive and reliable parameter estimated by the different lab/green house tests

5. Because the results of the different tests (lab, green house and field) are very highl correlated, the choice of the most suitable method does not (strongly) depend on the results, but cost effectivity and other (practical) arguments are more important.

6. Fodder radish D performed best. This genotype produced no egg masses in the different lab / green house tests (except for the second experiment of BBA) and was the only genotype in the field experiment white a final population density of *M. chitwoodi* statistically equal to the final population of black fallow.

7. In potato as proceeding test crop, all fodder radish genotypes, except FR C, gave a statistically equal tuber quality (TG-index) as black fallow.

Appendix 1

Frequency (%) histograms of plants classified to number of egg masses per plant for object A, B, C, D, E, Tomato, Potato and Fr Siletina in the greenhouse experiments carried out by PRI, HZPC and BBA (1 and 2). The number of plants per object in each experiment is mentioned under the object code in each histogram. The classification is

0: 0 egg masses / plant	16: 9-16 egg masses / plant
4: 1-4 egg masses / plant	32: 17-32 egg masses / plant
8: 5-8 egg masses / plant	>: >32 egg masses / plant