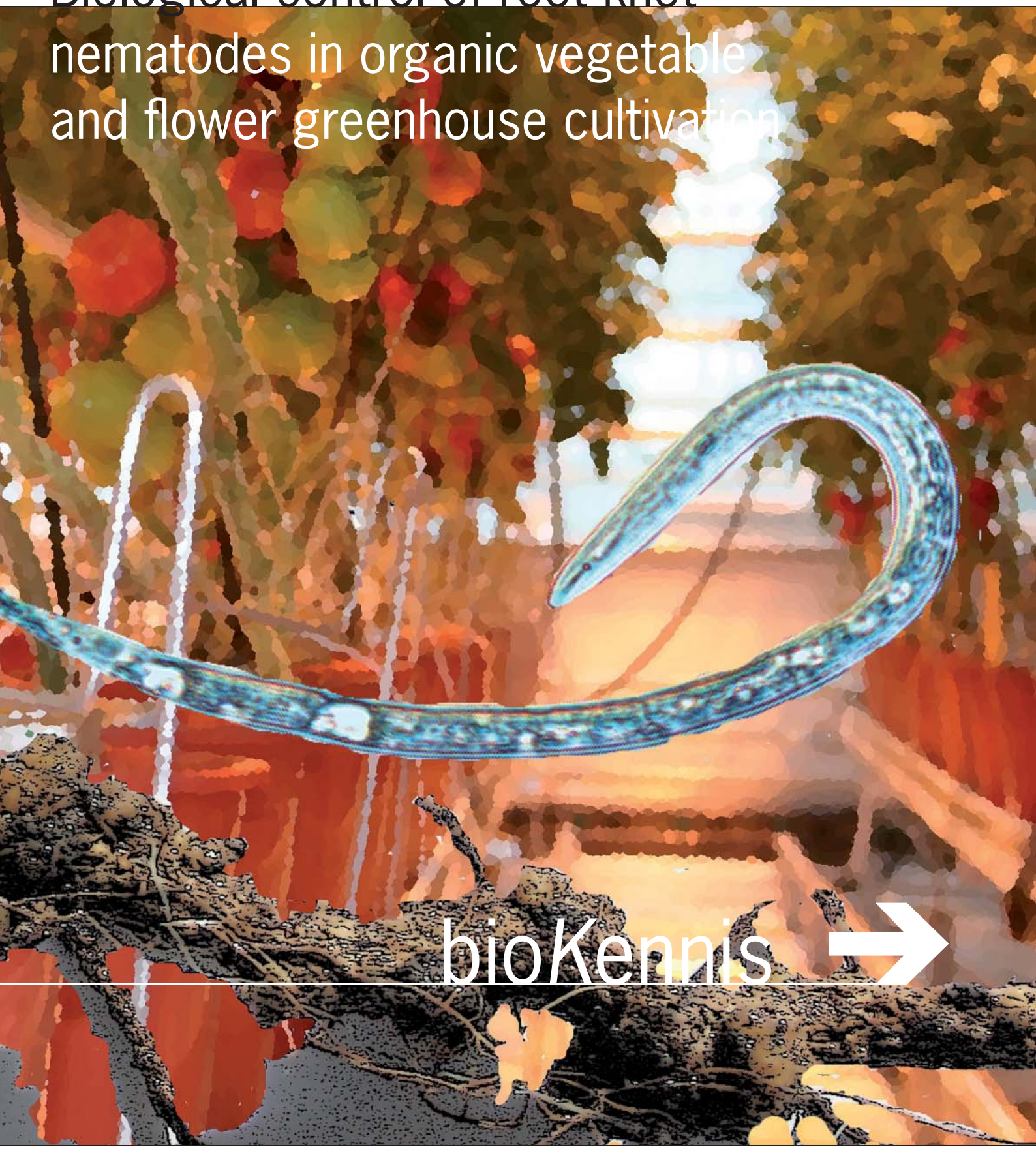
Biological control of root knot nematodes in organic vegetable and flower greenhouse cultivation



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Biological control of root knot nematodes in organic vegetable and flower greenhouse cultivation

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Report of a study over the period 2005 - 2010

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This research has been carried out in the context of the LNV (Netherlands Ministry of Agriculture, Nature and Food Quality) framework programmes System Innovation Protected Organic Farming BO-04-005 and BO-04-012 and BO-06-003 Innovation and Improvement Management of Closed Cultivations.

Ministry of Agriculture, Nature and Food Quality

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Most research on organic agriculture and food in the Netherlands takes place in the scope of the cluster Organic Farming, mainly financed by the Ministry of Agriculture, Nature and Food Quality. Bioconnect, the knowledge network for organic agriculture and food, is directing the research programmes (www.bioconnect.nl). Wageningen University and Research Centre and Louis Bolk Institute carry out most of the research activities. This report has been produced within this context.
The results of the various knowledge projects are presented on the website www.biokennis.nl. Questions and/or remarks about this research on organic farming and food may be mailed to: info@biokennis.nl. Suggestions for research may also be mailed to the Bioconnect windows on www.bioconnect.nl or a mail to info@bioconnect.nl.

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Preface

The Netherlands Ministry of Agriculture, Nature and Food Quality (LNV) has been financing nematode research in organic vegetable and flower cultivation in the context of the programmes System Innovation Protected Organic Farming: Vegetables, Flowers and Mushrooms BO-04-005 and BO-04-012 and BO-06-003 Innovation and management for plant health in contained cropping systems.

Research has been carried out in cooperation with, in particular, the organic vegetable growers Gebr. Verbeek, Fam. Baijens, F. de Koning, R. van Dijk, R. van Paassen and R. van Schie, A. Jonkers, and growers of organic flowers H. Cuppen, R. de Witt and F. van der Helm. The (already running) projects described here were discontinued in September 2006 by F. Zoon and C.J. Kok (Plant Research International) and continued at Wageningen UR Greenhouse Horticulture, Bleiswijk, The Netherlands. The projects were completed in 2009.

We thank researchers R. Berkelmans (Gebr. Verbeek) and T. Vink (Van Schie, Greenshields) for the close collaboration. We also thank Wageningen UR Greenhouse Horticulture, and Marc van Slooten, Wim van Wensveen, Laxmi Kok and Jan Janse in particular, and colleagues of Plant Research International (PRI) Peter Bonants for molecular detection of *Pasteuria penetrans*, Leo Poleij and Chula Hok-A-Hin for nematode and *Pasteuria* analysis, and PPO-BBF (Bulbs, Nursery Stock, Fruit) Gera van Os and Marjan de Boer for use of Topsoil soils, Astrid de Boer and Yvonne Elberse for use of mistifiers, and Frank van der Helm for assistance in organic flowers. Hendrik Terburg is acknowledged for aid with translating this manuscript. And we thank all growers of organic vegetables and flowers for their dedication and enthusiasm. The cooperation was a very pleasant one and we hope to continue this in future.

We have attempted to present a review of possibilities in order to arrive at a sustainable solution for root knot nematode control in soil-bound cultivation. We also tried to deal with the information about growers and holdings involved in the project in the most careful way. We have decided on the form of a book rather than a technical report of all experiments to improve readability for growers and other users for whom it is in fact intended. Details, such as experimental design and testing methods, have been omitted to improve readability. Questions about setup and details of the experiments can be addressed to the authors.

Research is ongoing in (organic) greenhouse horticulture and we are expecting new and/or improved options for biological control of root knot nematodes in the years ahead. The research activities in the LNV projects Bio-vital Greenhouse (BO-04), Soil Advisory System (BO-06) and Bio-Rotation Greenhouse (BO-04), in particular, offer perspective. These projects are focusing on the role of soil suppressiveness in organic greenhouse horticulture and in conventional chrysanthemum cultivation and options for management of suppressiveness, respectively. The Bio-rotation Greenhouse project also focuses on the further development of various (rotational) cultivation systems, of which the Baijens crop rotation system, as presented in this report, has been a first attempt. A follow-up study will be conducted towards different rootstocks with respect to different root knot nematode species (project Rootstock BO-04) and into optimisation of biological soil disinfestation (BSD; project within program LNV BO-04).

The authors,

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Bleiswijk, January 2010

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Summary

From 2005 to 2009, research was directed towards obtaining a solution for the damage caused by the root knot nematode *Meloidogyne* spp. in organic flower and vegetable cultivation. Soil steaming is often used and is an effective control method; this method, however, requires large amounts of energy and eliminates beneficial soil organisms. Our research therefore aimed at the development of alternative control options. The study focused on means as well as on methods, ranging from biological means and soil disinfestation to systems solutions such as use of crop rotation schemes. This project was financed by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV).

A large number of biological means were investigated; most, however, showed no or almost no effect. Only borium and some (legally) unauthorised plant extracts showed some level of control. The role of compost turned out to be promising. The production of root knot nematode suppressive compost, however, is not standardised and such compost is therefore not yet commercially available.

The bacterium *Pasteuria penetrans* from Japan was extremely effective against *M. javanica* and some populations of *M. incognita*. However, it is not yet being used in practice since it requires legal authorisation as crop protection agent and it is only effective against some root knot nematode species or populations.

At the moment biofumigation (*the use of toxic isothyocyanate gas in soil by incorporating e.g. Brassicacae*) as soil disinfestation appears to be unpredictable in terms of effectiveness. This may be caused by plant type, time of harvest and speed of use. In addition, Sarepta mustard (*Brassica juncea*) appears to be a good host for *Fusarium avenaceum*. Its use is therefore not recommended in, e.g., freesia and *Eustoma* spp. cultures as it is a known pathogen of cut flowers.

Biological soil disinfestation (*extracting oxygen from soil by means of incorporating easily decomposable material*) is labor -intensive but seems easier to standardize than biofumigation, thus increasing the chance of effectiveness.

In addition, the Baijens system appears to be an effective method to diminish crop losses caused by root knot nematodes. This system represents a spatial alternation of cucumber and alternative means of soil disinfestation such as biofumigation, biological soil disinfestation, black fallow and intercropping of antagonistic plants such as *Tagetes*. Rows are alternately planted with cucumber plantlets and an alternative soil disinfestation is used in between. Production loss is prevented by directing cucumber stems over intermediate rows, thus maximising production area.

It is concluded that a satisfactory and sustainable *one-option-fits-all* to prevent crop loss by the root knot nematode is currently not available. The answer lies in a combination of methods, depending on Melodoigyne species identity, crop type, growing system and soil. Ongoing projects, financed by the Netherlands Ministry of Agriculture, Nature and Food Quality (LNV) are focusing on soil suppressiveness in agricultural systems and offer a framework in which the interdependence of *Meloidogyne* species identity, crop type, growing system, soil type, and effectiveness of treatments can be studied.

Samenvatting (Dutch)

Gedurende de periode 2005 – 2009 is er onderzoek gedaan naar duurzame oplossingen voor gewasschade veroorzaakt door het wortelknobbelaaltje (*Meloidogyne* spp.) in the biologische teelt van bloemen en groenten. Op dit moment wordt grondstomen gezien als belangrijkste middel tegen wortelknobbelaaltjes. Grondstomen is effectief tegen wortelknobbelaaltjes maar kost veel energie en doodt nuttig bodemleven. Het onderzoek was daarom gericht op het ontwikkelen van alternatieve beheersingssystemen voor wortelknobbelaaltjes waardoor schade wordt geminimaliseerd en stomen overbodig wordt.

Er is gezocht naar zowel middelen als systemen, variërend van biologische middelen en grondontsmetting tot teeltsysteemoplossingen. Het onderzoek werd gefinancieerd door het ministerie van Landbouw, Natuur en Voedselkwaliteit (LNV).

Er zijn veel middelen getoetst maar het overgrote merendeel liet niet of nauwelijks een werking zien. Alleen borium en enkele niet-toegelaten plantenextracten lieten wel effectiviteit zien maar geen volledige bestrijding. De werking van compost is veelbelovend, maar het maken van aaltjeswerende compost is vooralsnog niet in the praktijk gebracht. De bacteriële bestrijder *Pasteuria penetrans* uit Japan was zeer effectief tegen *M. javanica* en enkele populaties *M. incognita*. Maar het ontbreken van wettelijke toelating en specificiteit van de stammen zijn redenen waarom deze bestrijder niet wordt gebruikt in de praktijk.

De effectiviteit van *Biofumigatie* (*onderwerken van bv. Brassicaceae-soorten waardoor isothiocyanaatgas vrijkomt*) als grondontsmetter blijkt vooralsnog onvoorspelbaar door de invloed van plantensoort, moment van oogst en snelheid van onderwerken. Ook blijkt sarepta mosterd (*Brassica juncea*) een goede waardplant voor *Fusarium avenaceum*. Hierdoor worden problemen zoals met *F. avenaceum* in de teelt van biologische Freesia en Lisianthus versterkt.

Biologische grondontsmetting (BGO; *o.a. zuurstof onttrekken aan de bodem door onderwerken organisch materiaal*) is arbeidsintensief maar lijkt makkelijker te standaardiseren dan biofumigatie waardoor de voorspelbaarheid van de mate van effectiviteit toeneemt.

Ook het Baijens teeltsysteem blijkt een effectieve methode om productieverliezen door aaltjes terug te dringen: Hierbij worden bedden afwisselend met komkommer beplant en over tussenliggende bedden heen geleid. In het tussenliggende bed is ruimte voor aaltjesbestrijding door inzet van aaltjesdodende planten of alternatieve grondontsmetting zoals braak, vanggewassen, biofumigatie of BGO.

Uit het onderzoek komt naar voren dat er op dit moment nog geen middel of methode voorhanden waarmee alle problemen kunnen worden opgelost. Op dit moment bestaat de oplossing vooralsnog uit een pakket aan maatregelen waaruit gekozen kan worden afhankelijk van doelpathogeen, gewas, bedrijfstype en bodemsamenstelling. Het lopend LNV onderzoek naar bodemweerbaarheid biedt een raamwerk waarin de invloed van deze factoren op the effectiviteit van de genoemde technieken verder wordt uitgediept en met elkaar in verband kan worden gebracht.

1 Nematodes

1.1 What are nematodes?

Eelworms or nematodes (Greek word '*nema*'= wire) represent one of the largest groups in the animal kingdom and are found virtually in all environments: not only in soil and water, but also in plants, animals and humans. Some 20.000 nematode species are known worldwide, half of which occur on land and in freshwater. About 1200 nematode species are found in the Netherlands of which some 100 species are plant-parasitic.

Most nematode species, however, are beneficial. Nematodes play an important role in the soil food web, e.g. in the decomposition of organic matter (de Ruiter *et al*. 1998). Other beneficial nematodes are insect parasites, some of which are sold as biological control agent. Examples are *Steinernema* and *Heterorhabiditis* species. Besides entomopathogenic nematodes and saprophytes there are carnivorous (predatory) nematodes such as *Mononchus* sp. and *Mylonchus* sp. that are predating on plant-parasitic nematodes such as *Meloidogyne*. Fungus-eating nematodes such as *Aphelenchus avenae* and *Aphelenchoides* spp. graze on (plant-parasitic) fungi such as *Fusarium* spp., *Botrytis cinerea, Pyrenochaeta lycopersici* (corky root) and *Verticillium dahliae* (Hasna *et al*. 2007). They play an important role in the soil suppressiveness of plant-parasitic fungi (see §4.6).

Plant-parasitic nematodes need living plants to feed on and for reproduction. Depending on the place where root nematodes are found they can be subdivided into:

1. Ectoparasitic (living outside the plant)
2. Semi-endoparasitic (partially living within the plant)
3. Endoparasitic (fully living within the plant)

Of these, the endoparasitic nematodes are most important in the organic cultivation of vegetables under glass. Root nematodes start searching for roots and in the soil they can actively move a distance of about one metre per year. Besides the virus-transmitting nematodes (such as the Trichodorids), which are living ectoparasitically, the endoparasitic nematodes cause the most severe problems. These nematodes are seriously ruining plant tissue because they move between and through the cells or they restrict the root function through hormonal effects and by forming food cells. The endoparasitic nematodes are divided into two groups: migrating nematodes (such as leaf, stem, root lesion and root necrosis nematodes) and sedentary nematodes (such as root knot and cyst nematodes).

Root lesion nematodes (*Pratylenchus penetrans*), which have a wide host plant range, including Solanaceae, endive and lettuce, are mainly restricted to the light soil types from sand to loam. Problems with cyst nematodes and root lesion nematodes in organic vegetable cultivation are unknown until now. Virus transmitting root nematodes, which are in particular occurring in lighter soils, neither play –insofar as known- a role in organic vegetable cultivation.

Experience from the past learns that especially root knot nematodes (*Meloidogyne* spp.) play a significant role (Table 1) in organic greenhouse vegetable cultivation. Other nematodes cannot be ruled out but root knot nematodes play the major role. Besides root lesion nematodes, root knot nematodes are also most common in flower cultivation.

Table 1. Plant parasitic nematodes per 100 cc soil (mixed sample) on 20 organic greenhouse vegetable holdings (sampling August 2008). j = juvenile, m = male, f = female. Root knot nematodes (Meloidogyne spp.) in bold print.

Holding		**L**	**G**	**B**	**AG**	**Q**	**AF**	**D**	**O**	**AE**	**C**
Paratylenchus spp.†	j	-	-	-	-	-	-	-	-	-	18
Paratylenchus projectus	v	-	-	-	-	-	-	-	-	-	-
Tylenchidae *	j	38	14	81	100	214	105	48	8	65	111
Tylenchidae	m	-	-	-	17	-	-	-	-	-	-
Tylenchidae	v	-	-	-	17	-	-	105	-	-	-
Criconematidae	j	-	-	-	-	-	-	-	-	-	18
Dolichodoridae	j	-	-	-	-	-	-	-	-	-	-
Hemicycliophora spp.	j	-	-	-	-	-	-	-	-	-	-
***Meloidogyne* spp.**	**j**	**-**	**596**	**1663**	**17**	**9394**	**105**	**355**	**25**	**52**	**886**
Meloidogyne chitwoodi	**j**	**-**	**41**	**-**	**-**	**-**	**35**	**-**	**-**	**-**	**-**
Meloidogyne fallax	**j**	**-**	**14**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Meloidogyne hapla	**j**	**-**	**244**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**498**
Pratylenchus spp.	j	-	-	-	33	-	-	-	-	-	-
Pratylenchus neglectus	v	-	-	-	-	-	-	-	-	-	-
Pratylenchus penetrans	v	-	-	-	-	-	-	-	-	-	-
Rotylenchus spp.	j	-	-	-	-	-	-	-	-	-	-
Tylenchorhynchus spp.	j	-	14	-	17	-	-	10	-	-	37
Tylenchorhynchus dubius	m	-	-	-	-	-	-	-	-	-	-
Tylenchorhynchus dubius	v	-	-	-	-	-	-	19	-	-	18
Longidorus spp.	j	-	-	-	-	-	-	-	-	-	-

		AH	**N**	**E**	**Z**	**F**	**AA**	**AD**	**AI**	**H**	**R**
Paratylenchus spp.	j	-	-	-	24	-	-	-	-	-	-
Paratylenchus projectus	v	-	-	-	8	-	-	-	-	-	-
Tylenchidae	j	63	57	70	16	68	148	91	284	122	294
Tylenchidae	m	21	-	-	16	-	-	13	-	11	38
Tylenchidae	v	21	-	18	8	-	15	13	-	-	38
Criconematidae	j	-	-	-	-	-	-	-	-	-	-
Dolichodoridae	j	-	-	-	-	-	-	143	-	-	13
Hemicycliophora spp.	j	-	-	-	-	-	89	-	-	-	-
***Meloidogyne* spp.**	**j**	**211**	**228**	**35**	**8**	**1902**	**44**	**13**	**-**	**44**	**-**
Meloidogyne chitwoodi	**j**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Meloidogyne fallax	**j**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Meloidogyne hapla	**j**	**63**	**-**	**-**	**8**	**476**	**30**	**-**	**-**	**-**	**-**
Pratylenchus spp.	j	-	-	-	-	-	-	-	-	-	-
Pratylenchus neglectus	v	-	-	-	24	-	-	13	-	-	-
Pratylenchus penetrans	v	-	-	-	16	-	-	-	-	-	-
Rotylenchus spp.	j	-	-	-	-	-	30	13	-	-	-
Tylenchorhynchus spp.	j	-	-	-	56	-	15	-	-	-	-
Tylenchorhynchus dubius	m	-	-	-	8	-	-	-	-	-	-
Tylenchorhynchus dubius	v	-	-	-	8	-	-	13	-	-	-
Longidorus spp.	j	-	-	-	-	-	-	13	-	-	-

† *spp. means that several species are possible.*

* *Tylenchidae juveniles are difficult to identify and they are therefore often classified under the family name.*

1.2 Root knot nematodes

The number of root knot nematodes may on an average organic greenhouse holding reach levels up to thousands per 100 g soil. The largest problem in organic vegetable cultivation under glass is the root knot nematode *Meloidogyne incognita*. This nematode flourishes with high temperatures, lays eggs in the roots of all important fruit vegetable crops and is especially problematic in cucumber and tomato. Knots or galls are formed after nematodes have laid eggs within the roots, which disturbs nutrient uptake resulting in poor crop development.

The eggs within the roots present the largest problem. Nematodes can in this way survive for a long time and they are difficult to control. Apart from the *incognita* nematode, other root knot nematodes are also present in most greenhouse soils in the Netherlands, such as *Meloidogyne hapla*, *M. javanica* and *M. hispanica* (see also Table 4). These nematodes may also cause damage to the crop and are difficult to control.

Root knot nematode infestation is generally easily recognised by the presence of root knots (Photo 1). Depending on the crop, root knot nematode species and age of the infestation, smaller or larger root knots can be found. The root knots induced by *M. hapla,* e.g., are generally branched and only a few millimetres thick, whereas an infestation by *M. incognita* more often than not causes knots up to 2 cm.

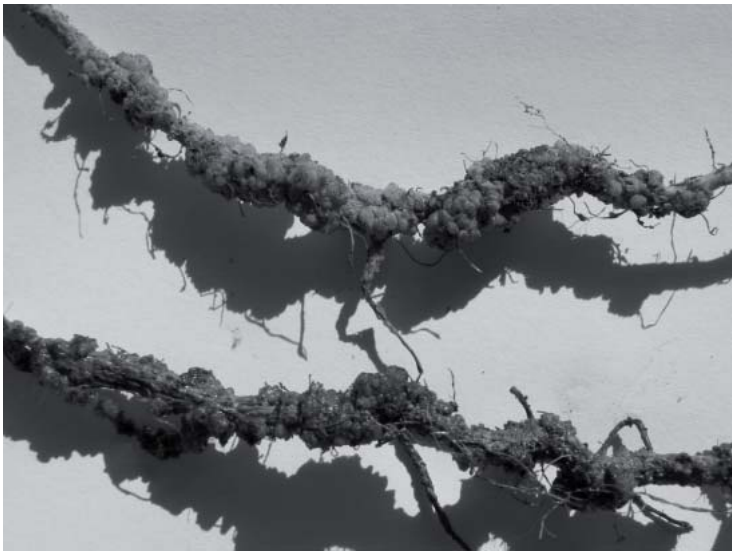


Photo 1. Root knots caused by the root knot nematode Meloidogyne spp.

Root knot nematodes (Meloidoginidae family) form a close entity within the Tylenchina - Tylenchomorpha. The Tylenchomorpha group includes the largest and most important groups of plant-parasitic nematodes, such as root knot nematodes (Meloidogynidae), root lesion nematodes (Pratylenchidae), tylenchide nematodes (Tylenchidae), Hoplolaimidae and Heteroderidae (including cyst nematodes) (Holterman *et al.* 2007).

Root knot nematodes are found all over the world and have a very wide host plant range. This very wide host plant range in particular makes them difficult to control.

Root knot nematodes (*Meloidogyne* spp.) belong to the endoparasitic nematodes and penetrate the roots of the plant where they affect plant tissue and restrict root functioning. This results in a reduced sap flow to aboveground parts resulting in the plant starting to '*wilt*'. Root knot nematodes take many different shapes and sizes (Table 2). The southern root knot nematode (*M. incognita*) is a problem in organic greenhouse cultivation of vegetables such as cucumber, tomato and sweet pepper. Other nematodes found in these greenhouses are the northern root knot nematode (*M. hapla*), the peach root nematode (*M. hispanica*), the Javanese root knot nematode (*M. javanica*) and the maize root knot nematode (*M. chitwoodi*). This last nematode causes major economic damage in several crops

in the Netherlands, including potatoes, peas, carrots and scorzonera. This is why the maize root knot nematode has had the quarantine status (*Q* status) since May 1998. This means that all propagation material, such as seed material, planting material, tubers and bulbs, must be free from this nematode.

Table 2. Root knot nematode species: English- and scientific names.

Root knot nematodes	
False Columbia root-knot nematode	*Meloidogyne fallax*
Barley root knot nematode	*M. naasi*
-	*M. maritima*
-	*M. ardenensis*
Coffee root knot nematode	*M. exigua*
-	*M. artiellia*
Maize root knot nematode	*M. chitwoodi*
Northern root knot nematode	*M. hapla*
Olive root knot nematode	*M. lusitanica*
Seville root-knot nematode	*M. hispanica*
Rice root knot nematodes	*M. graminicola, M. oryzae*
-	*Nacobbus aberrans, Meloidogyne arenaria*
Peanut root knot nematode	
peanut root-knot nematode	*M. incognita, M. javanica*
-	*M. duytsi,*
-	*M. kralli*

(From: Karssen et al. 2001; http://www.journal-of-nematology-style-guide.org/common_names.html).

The *Meloidogyne enterolobii* (syn. *M. mayaguensis*) species has recently been found in the Netherlands in a batch of imported roses (PD (Plant Protection Service), February 2008). It is known that *M. enterolobii* easily breaks down resistance and has a wider host plant range than its congeners. This is why it has been given the common name quarantine species 'Number 1'. This nematode has first been described in aubergine in Puerto Rico. In 2001 this nematode was first found in North America – Florida. It has also been found in Cuba (1989), South-Africa (1997) and West Africa (1994 – 2000), Guadalupe and Martinique (2000), Malawi and Tobago-Trinidad (2000), Brazil (2001) and France (2002). It has recently been found in two greenhouses in Switzerland (February 2008). It is as yet unknown whether this nematode is present in greenhouses in the Netherlands.

1.3 Life cycle of root knot nematodes

Root knot nematodes are reproducing within the root. By manipulating the plant they can produce feeding cells ('*giant cells*' or syncytia) and reproduce. The females deposit the eggs outside the body in a gelatinous mass, the so-called egg mass (Photo 2). Such an egg mass may contain up to 1000 eggs. This causes the characteristic root knots. The eggs hatch and the free-living J2 nematodes leave the root to penetrate yet another plant. This stage is active for about a week, depending on soil temperature.

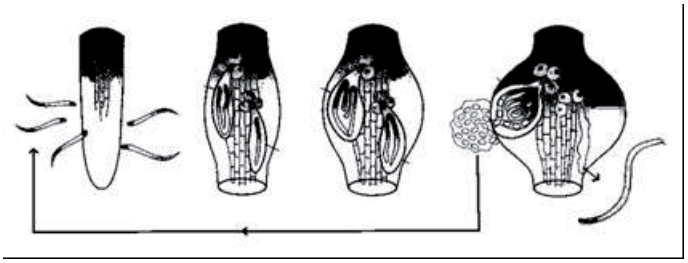


Photo 2. *Life cycle of the root knot nematode (Meloidogyne spp.). The first drawing depicts a root tip with the so-called free-living (J2) root knot nematodes. The second drawing shows root knot nematodes in the swelling root (J3). The nematodes in the third drawing have reached the J4 stage. The last drawing shows the bursting of the nematodes and an egg mass protruding from the root. The arrow indicates new J2 larvae leaving the eggs.*

The egg masses, mainly in root debris, remain vital for a longer period of time. Their dormant presence may, again depending on soil temperature, run up to years. The damage to the roots causes less efficient transport of water and nutrients to the aboveground parts of the plant, which results in growth suppression.

The life cycle of root knot nematodes differs per species and depends on soil temperature and – to a lesser extent - nutrition. Table 3 presents the length of a life cycle (in days) in relation to soil temperature of root knot nematodes that may be present in greenhouses with organically grown vegetables. At 18°C, e.g., the life cycle of *M. incognita* takes over seven weeks, while this is only 3.5 weeks at 27°C. These data enable calculation of the *temperature threshold* for each species and the *temperature sum* required above that threshold. This enables calculation of the effect of any temperature course.

Table 3. *Life cycle of root knot nematodes in relation to soil temperature (derived from Ploeg & Maris 1999). The given temperature sum above the threshold value is required for the appearance of the first J2. This temperature sum must be about doubled to reach the maximum number of progeny.*

	Life cycle (days)					
	Soil temperature				Temperature-	Temp.sum
Meloidogyne sp.	18 °C	21 °C	24 °C	27 °C	threshold (°C)	above threshold
M. arenaria	54	36	27	21	12.1	318
M. hapla	56	43	35	29	8.3	545
M. incognita	51	37	29	24	10.1	404
M. javanica	69	43	32	25	12.8	357

Root knot nematodes are hatching in the second juvenile stage (J2) and start searching for roots in soil. Only this stage is capable of infestation. The length of a J2 ranges from 380 to 460 µm (approx. 0.4 mm) with a diameter of approx. 14 µm. A J2 penetrates the root in the root elongation zone just behind the root cap and moves, between the cells, to the young vascular bundles where it induces a feeding site, consisting of giant cells and phloem bundles. These serve as food source for the developing nematode. The adult male or female stage is formed after three moultings, i.e., a J2, via the J3 and J4 stage.

A female is oval to spherical (540x800 µm) and milky white, and has a sedentary life style. A male is, contrary to the female, eel-shaped and leaves the root, possibly to mate. Many root knot nematodes, however, reproduce parthenogenetically, i.e., asexually, thus without mating. Because the swollen stages (J3, J4 and ♀) are found in the roots and cannot move, these stages will never be found freely in soil samples.

Females deposit the eggs outside the body in a gelatinous substance, the so-called egg mass. This provides the eggs with a reasonable protection against unfavourable conditions. An egg mass contains about 300 - 500 eggs but outliers up to thousand eggs are possible. Extra cell division results in the formation of root knots or galls on the roots which may vary in size and shape depending on the nematode species. When the root knots are only a few mm in size, virtually all eggs will be deposited outside the knot. In case of larger root knots many egg masses will be deposited within the knot itself. The size of the knots usually increases with the age of the infestation. Knots with a thickness of two cm are no exception in tomato and cucumber. The egg passes through an embryonic development which results in a first-stage-juvenile (J1). This remains in the egg and moults, after which the nematode hatches from the egg as a J2.

1.4 Identification of root knot nematodes

The aboveground symptoms of nematode infestation often take the shape of growth retardation, deficiency symptoms, wilting, and in the most serious case the plant dying off. Nematodes, however, are not the only pathogens that may cause such symptoms; these may also be caused by fungi such as *Phytophthora* spp. and plant- parasitic *Fusarium* spp. The crop needs further analysis or sampling to identify the true cause.

Use of a spade is desirable for root inspection. When the roots would simply be pulled out of the soil, many roots will be left in the soil which means that a difficult discernible infestation is easily overlooked. Experience learns that no nematode-free spots can be found in older greenhouses in which crops have been grown for years without steaming. A wide variation in densities, however, exists. This needs to be taken into account in particular in field experiments. This can be done by including the number of nematodes before and after treatment in the experimental analysis and intensive sampling.

Not all nematodes, however, leave such clear root symptoms as root knot nematodes. This means that it may also be necessary to carry out diagnostic samplings to identify the cause. This can be done by collecting soil and roots from the edge of a poor spot and at some metres distance to be analysed for pathogens. Comparison of both results gives an indication of the pathogen that may be causing the growth retardation. Several pathogens at the same time (disease complex) are possible as well. Diagnostic sampling is also required when it is unknown which root knot nematodes species are present. Nematodes and fungi can at the moment also be identified via *DNA* testing (Figure 1).

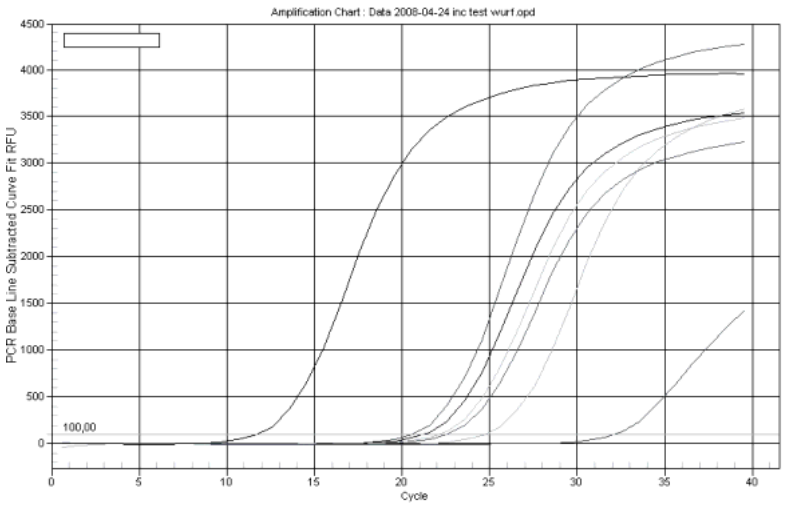


Figure 1. *Typical DNA qPCR graph (From: Blgg AgroXpertus, Wageningen, the Netherlands) with horizontally the number of cycles (time) of the polymerase chain reaction and vertically the amount of formed DNA marker for M. incognita. This enables estimation of the amount of M. incognita in the sample. The different lines represent markers for M. javanica, M. incognita and markers representing tropical species generally. The earlier the curve starts, increasing amounts of DNA of that species is present in the sample.*

1.4.1 Species identification

Identification of the plant-parasitic nematodes that are present on a holding or in a greenhouse section is an essential link in the nematode control strategy (Molendijk 1999). This forms the basis for taking effective measures. This certainly applies for the preparation of a good cropping scheme because resistances and tolerances have everything to do with the communication between plant and nematode, back and forth, which communication is often species-dependent. Large differences in susceptibility of a crop to various species of nematodes are more the rule than the exception. This is also true for damage-sensitivity. Identification is also desirable for opting biological control by means of fungi and bacteria. The effectiveness of biological control is in many cases determined by the species of the pathogenic nematode. A larger role of heat-demanding species such as *M. incognita* and *M. javanica* is to be expected at higher heating levels, such as in greenhouse horticulture, in comparison with the intermediate species such as *M. hapla* that are particularly flourishing in cold greenhouse cultivations.

Soil sampling per greenhouse section is a good method to establish which species are present. This enables a crop to be selected for each section, depending on the nematode species present. Current knowledge about the presence of plant-parasitic root nematodes on the holdings is often insufficient. In most cases it is known that root knot nematodes are present but reliable information about species is lacking. Let alone that something would be known about the presence of other plant-parasitic root nematodes.

Not all laboratories are capable to identify *Meloidogyne* species.

Species identification can be based on:

- The morphology of juveniles and females but this is rather specialistic and labour-intensive.
- A second method consists of an analysis of proteins (isozymes) from the females. Both methods are usually carried out with 20 individuals; this gives a reasonable chance that admixture of 5-10% of a different species will be noticed.
- The most recent (molecular) methods are based on the identification of species-specific pieces of DNA via an accumulation technique (PCR or taqman-PCR); these techniques are currently enabling identification of all important *Meloidogyne* species and admixtures of 1-5% of a different species show up (Figure 1, Table 4).

Table 4. Broad analysis of root knot nematodes on 12 holdings with molecular DNA qPCR detection (October 2008). Holdings are presented according to the Biokas 2005-2008 code. Numbers are given per 100 cc soil. Meloidogyne spp. indicates that root knot nematodes could not be identified (molecular detection for various other species such as M. enterolobii syn. M. mayaguenensis is not yet available).

Holding	*M.chitwoodi*	*M.fallax*	*M.minor*	*M.naasi*	*M.hapla*	*M.javanica*	*M.incognita*	*Meloidogyne spp*
AG	-	-	-	-	1	2000	-	-
AH	-	20	-	14	1244	-	460	-
B	-	1	-	-	2	-	3413	-
C	-	-	-	-	2800	700	1400	-
D	-	-	-	-	4	0	1455	-
E1	-	-	-	-	-	15	0	-
E2	-	-	-	-	2	1	5	-
G	-	662	-	-	9308	5	10	-
N	-	-	-	-	-	-	455	-
O	-	-	-	-	-	-	-	10
F1	-	2	-	-	167	-	1000	-
F2	-	-	-	-	11110	-	-	-
F3	1	-	-	-	7	-	350	5350
F4	-	-	-	-	1	-	6980	-

F1 = sweet pepper - grower F; F2 = tomato - grower F, F3 = cucumber - grower F, F4 = tomato - grower F.

1.4.2 Biotype identification

More detailed identification may sometimes be desirable for an accurate prediction of the host plant range of the nematode, viz. at biotype level. A biotype within a nematode species may relate to a deviating host plant range (biotype = physiological race), a deviating interaction with a resistance gene (biotype = pathotype), or, e.g., a deviating reproductive system. Four physiological races are known of *M. incognita* and two of *M. arenaria*. *M. hapla* has two biotypes related to the reproductive system but probably also to the host plant range. All these biotypes are probably infesting tomato and other Solanaceae but there is a difference for other plant families. This means that it may for certain crops be desirable to determine the host plant status per holding but this is a labour-intensive procedure.

1.5 Soil type, water and fertilisation

Soil type gives an indication of the nematodes that may be present. Root knot nematodes in particular occur on lighter soil types (Norton 1978), but heavy clay is possible as well. *M. incognita,* e.g., is present on a holding with 46% silt where is reproducing well.

Water content of the soil is also important because root knot nematode eggs are suffering from osmotic stress in dry soil. The larvae in the eggs die when the eggs become too dry.

Inorganic salts are also known to play a role in root systems attracting and repelling root knot nematodes. Simple salt ions such as K^+, NH_4^+, Cs^+, NO_3^-, and Cl^- are repelling J2 root knot nematodes. It has been argued before (*cf.* Sudirman 1992) that salts, and NH_4^+ in particular, can get into eggs and juveniles (via osmosis) and inhibit energy production (ATP) resulting in the nematodes dying. Castro *et al.* (1990) found that the anion NO_3^- is most repellent, followed by $C1^-$, Br^- and I^-, respectively. For the cations they arrive at the order, from strongly to slightly repellent respectively: K^+, CS^+ and $NH4^-$. They also consider the chloride and nitrate salts of the last two cations as repellent (see also Sudirman 1984). Phosphite fertilisation (HPO_3^{2-}) in the form of H_3PO_3 probably reduces root knots (*M. maryland*) in cereals by stimulating the systemic resistance of the plant (Oka *et al.* 2007).

2 Problem description

Research on organic greenhouse fruit vegetables and flowers in the period 2006 - 2009 focussed on finding a sustainable solution for crop damage caused by soil-bound pests and diseases, in particular the root knot nematode *Meloidogyne* spp. This nematode may cause up to forty per cent yield loss in soil-bound organically grown crops such as tomato, cucumber, freesia and alstroemeria. In 2007 the root knot nematode was among the top 10 of the most-feared pests and diseases in the organic greenhouse cultivation of vegetables. This top 10 list has been drawn up on the basis of a survey held among growers in November 2007 (Table 5).

There are various causes for root knot nematodes being a major problem in soil-bound greenhouse crops. The intensive cropping method must be mentioned first. Especially in heated cultivations, the soil hardly gets time to recover via a natural decrease of these nematodes. It often takes less than a month after the old crop has been cleared before a new crop is planted.

Table 5. Top 10 pests and diseases in organic vegetable growing under glass, ranked from important to less important.

	Scientific name	English name
1	*M. incognita*	Southern root knot nematode
2	Other nematodes, such as *M. hapla, M. javanica*	Northern root knot nematode, Javanese root knot nematode
3	*Sclerotinia*	Sclerotinia disease
4	*Verticillium*	Wilting disease
5	*Pyrenochatea*	Corky root rot
6	*Pythium*	Pythium root and stem rot
7	*Fusarium*	Fusarium damping off
8	Millipeda	Millipedes
9	Isopoda	Wood lice
10	Symphyla	Centipedes (root)

Various methods and products of biological origin have been investigated in this study. These can be used on a wider scale, such as in the conventional cultivation of several soil-bound flower and vegetable crops. Examples are: alstroemeria, chrysanthemum, freesia, lisianthus, radish, lettuce. Products and methods of natural origin may also offer a sustainable alternative for pest and disease control in conventional cropping. Soil-bound greenhouse cultivation in the Netherlands covers about 2100 ha (Table 6), with 1625 ha ornamentals (chrysanthemum, freesia, alstroemeria, lisianthus, amaryllis, summer flowers, lily, other) and 475 ha vegetables (lettuce, radish, soft fruit, other). Economically, chrysanthemum and freesia are the most important soil-bound crops under glass (Table 6).

Table 6. Area of the economically most important soil-bound crops under glass in the Netherlands.

Crop	Area (ha)
Chrysanthemum	566
Bio-vegetables	70
Alstroemeria	93
Freesia	155
Lisianthus	40

Greenhouse cultivation does not have the same possibilities as field cultivation. A few summer months without vegetables or 'black fallow', or biological soil disinfestation works wonders. This is, however, an unmentionable option for greenhouse cultivation. Greenhouse cropping is expensive which necessitates non-stop year-round cropping for the organic grower. Chemical pesticides, insofar as these are still legally permitted, may not be used. And there are no active biological products. A crop rotation of cucumber, tomato and sweet pepper is of no use because none of these fruit vegetables is fully resistant. Expansion of the rotation with a different – fully resistant – crop is an option. But cultivation system, product grading and level of specialization is restricting the choice, while the demand for other organic-products such as flowers is often (still) too low. Targeting new markets would cost the grower a lot of time and effort.

2.1 Organic vegetable cultivation

Of the organic fruit vegetable crops, cucumber is under most pressure because resistance is lacking and nematode control has hardly been developed. But tomato and sweet pepper cultivation is under pressure as well. Resistance, insofar as present, is broken at high population densities of root knot nematodes and high temperatures. Complete resistance is for the time being not yet possible (Bouwman-van Velden & Janse 2009).

2.1.1 Costs and production loss

Until now, soil steaming (Runia 1992) is considered the most effective remedy but this costs a lot of energy, is expensive (Box 1) and eliminates a large part of soil life. This is why many growers consider steaming as unfit for an organic cultivation system. Growers do increasingly refrain from soil steaming, however in case of increasing crop damage they feel it is the only remedy to deal with soil pathogens.

BOX 1 Costs and production loss by root knot nematodes

Root stock (costs and production loss)

Damage caused by	**Euro per square metre**			
	Cucumber	Sweet pepper	Tomato	**Rotation**
Yield loss nematodes	10-20%	>5%	10-15%	10-20%
Yield loss rootstock	Extra yield** ***	0-20% yield loss + 1 euro grafts	Extra yield**	
Costs steaming (once per 2 years) *	1.50	1.50	1.50	1,50
Costs investment in steam drainage[2]	0.35	0.35	0.35	0,35
Total	1.85 + 10-20% loss	2.85 + 5-15% loss	1.85 + 10-15% loss	
Yields without nematodes (estimated, kg)	55 – 65	25 – 30	50 – 55	
Yields (Euro)	55.= – 65.=	60.= – 70.=	65.= – 75.=	
Relative costs nematodes related to yields	15 – 25 %	15 – 25 %	15 – 20 %	
Damage / m^2	8.= – 15.=	4.00 – 7.50	7.50 – 10.=	

** Steaming is fully ascribed to nematode problems.*

*** Extra costs grafted plant are compensated by wider planting (K. Cornelissen; PCG, pers. comm..)*

*** Extra yield in relation to cultivation in soil with nematodes – grafting of cucumbers as such results in a production loss of 5-10%.*

From: Vermeulen et al. 2008.

[2] Closed system with two tubes per 3.20 m = € 2.50/m2 assuming 7% depreciation, 1% maintenance and 6% investment costs = € 0.35/m^2.

2.1.2 Three holding types and soil steaming

Greenhouse organic cultivation in the Netherlands can broadly be classified into three types of holdings, i.e., fruit vegetable cultivation year-round 1:2 or 1:3, fruit vegetables alternated with leaf vegetables in the winter period, and cold greenhouse or slight heating (air heating) with a wide crop rotation. There are no growers of year-round leafy crops in the Netherlands. Especially year-round growers of fruit vegetables (cucumber, tomato, sweet pepper) face the largest problems with root knot nematodes such as *Meloidogyne incognita*.

Soil originating from twenty organic holdings belonging to the above-mentioned categories have in 2008 been analysed for a large number of factors, such as abiotics; nematode diversity, streptomycete and pseudomonad community; total fungal and bacterial biomass. Figure 2 shows an analysis from this study in the form of an ordinance analysis (*principal component analyses*; PCA). This enables summarising of the correlation between several measurements in one glance, the so-called ordinance graph. This shows that in particular the "cold" greenhouse holdings have the highest diversity of Pseudomonad species in the soil. This group consists of several types of bacteria that may play a role in the suppressiveness of the soil towards pests and diseases but also towards pathogens, such as those causing bacterial rot *(Pseudomonas cichori)* and lettuce drop *(Sclerotinia sclerotiorum)* in lettuce and leeks (Vanhouteghem *et al*. 2006). These soils also contain a relatively large amount of fungal biomass and a complex food web; this can be concluded from the differences in the nematode community (Figure 2). As already described in §1.1.1, nematodes form an extremely diverse group, including entomopathogenic, saprohagous and predatory nematodes. These nematodes fulfil different roles in the food web and can therefore be considered as representative of the diversity of the food web.

The opposite situation can be found in the soil of the holdings with year-round fruit vegetable cultivation (Figure 2). The soil of these holdings contains more bacterial biomass, a simpler food web structure with relatively high nitrate concentrations.

The holdings with a rotation of fruit and leafy vegetables take up the intermediate position (Figure 2). The largest diversity of Steptomycetes is found in this group. These are bacteria that may in particular be involved in soil suppressiveness towards pests and diseases, including root knot nematodes (Da Silva Sousa *et al*. 2006). They are in particular responsible for the degradation of dead plant debris and are known for the production of substances against (plant-parasitic) bacteria and fungi.

Finally, the analysis shows that especially holdings with year-round cultivation of fruit vegetables experience problems with *Meloidogyne* spp. It is noteworthy that the soil of these growers is also characterised by a higher nitrate concentration in the soil in comparison with holdings with cold greenhouse cultivation. Year-round growers of fruit vegetables are more frequently using soil steaming; this may have a negative effect on organisms such as pseudomonads, which play an important role in denitrification. This results in nitrate accumulation in the soil. The above theory, incidentally, also explains the low diversity of pseudomonads in these soils: Pseudomonads are known to be temperature-sensitive. This may imply that soil steaming does indirectly cause the high nitrate concentration by removing pseudomonads from the soil system and stopping denitrification. The nitrate concentrations found in the study ranged from 0.9 to 7.9 mmol, and are as such not high.

Nitrate as well as ammonium may affect pathogens, antagonists as well as growth and resistance of the plant. When denitrification dominates and ammonium values are high, this has a negative effect on root knot nematodes. But nitrate can, e.g. in tomato, also induce extra vigour which makes the plant more tolerant towards root knot nematodes (Spiegel *et al*. 1982) and even repellent (Castro *et al*. 1990). In addition, pseudomonads may, e.g., be closely involved in denitrification processes as a result of which they may have an indirect repellent effect on root knot nematodes.

This means that an optimum balance between nitrate and ammonium is best for optimum plant growth and for stopping syncytia formation, i.e., a giant cell as food source for root knot nematodes and that soil steaming plays a negative role in this process.

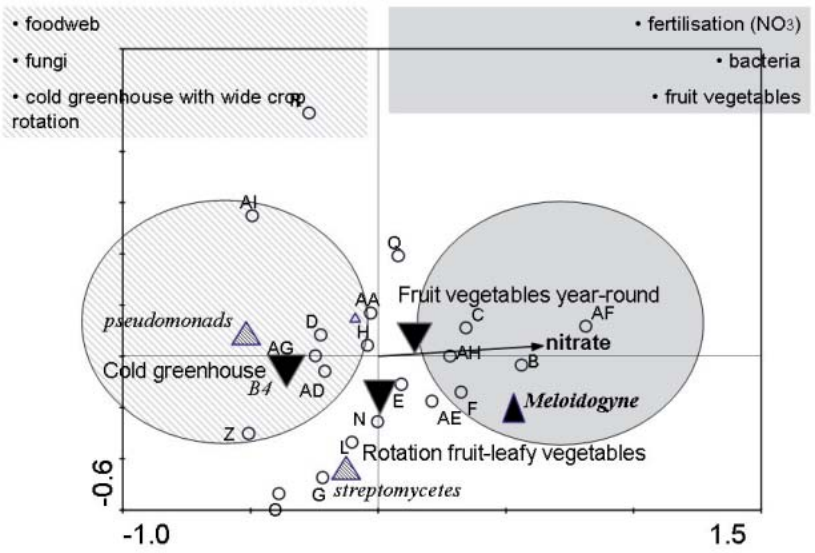


Figure 2. *Ordinance diagram (PCA) based on soil functions and bacterial diversity; Pseudomonads and Streptomycetes in soil of 20 organic greenhouse holdings. The horizontal axis explains 97.3% of all variation and mainly represents an increase in nitrate (black arrow). Triangles (▲) indicate the position of the type of holding (a.) fruit vegetables year-round, (b.) cold greenhouse with wide crop rotation, and (c.) rotation of fruit vegetables and leafy vegetables in winter. Letters represent holdings with organic greenhouse vegetables. Points that are closely together show a positive correlation, the correlation increases with an increasing distance from the intersection of both axis (zero). Points on the same line but in the opposite direction show a negative correlation (increasing the one results in a decrease of the other). Because the horizontal axis explains most of the variation, the points close to this axis are the most important ones.*

2.2 Organic flower cultivation

Root knot nematodes (*Meloidogyne* spp.) present one of the largest bottlenecks in organic and soil-bound integrated cultivation of flowers under glass. *Fusarium avenaceum* is another important cause of plant loss, in crops such as freesia and lisianthus. Loss caused by soil pests and diseases in organic flower cultivation may rise to 15%. Also here, soil steaming is until now the most effective remedy but this is very expensive and growers consider it as unfitting in an organic cultivation system.

Freesia, amaryllis and lisianthus were until recently the most important crops but the decreasing demand in combination with the increasing productions costs of flowers under glass caused an enormous reduction in the number of organic greenhouse holdings in recent years.

A very extensive review of nematode host plants and options for organic rotational flower cultivation has been prepared together with PPO-BBF, Lisse, the Netherlands (Box 2, see Annex V for a complete list). For further information about assortment, host plant status for nematodes, and options for rotation systems we refer to Van der Helm *et al.* (2008; 2009).

BOX 2 Host plant status and crop rotation in summer flowers

The report as well as the brochure present a review of the host plant status of (organic) summer flowers for nematodes. Host plant status means whether nematodes can reproduce on the crop. Damage and host plant status are not the same. The information in the table (Annex V) is based on a literature study of Dutch and international research. These results therefore require some reservation. Relatively little is known about nematodes in perennial plants because little research is being carried out and the assortment is large. This makes it impossible to prepare a detailed review as has been made for, e.g., arable farming and bulb crops (www.nematodesschema.nl). The review in this brochure is based on plant genus but differences between species and even cultivars are quite well possible. The complete list with host plant status, where possible at cultivar level, can be consulted via internet www.ppo.wur.nl and in Annex V.

Whether nematode problems arise in summer flowers and perennial plants depends on survival, spreading, and reproduction of the nematodes in the soil and on the infestation of the fields by the introduction of nematodes. Each nematode control method in soil and plant material has its limitations and disadvantages; this makes prevention always better than curing. The best method consists of the combination of several measures in a nematode control strategy (NCS). It is described on www.nematodesschema.nl how such a strategy can be drawn up. Measures to prevent problems with nematodes must be implemented on the total holding, start with healthy, nematode-free soil and healthy propagation material and end with a good check of the plants that leave the holding (*From* Van der Helm *et al*. 2008, 2009).

2.3 Structure of the report

Organic systems are aiming at a sustainable cultivation of vegetables and flowers. An agricultural may only be referred to as organic if the production process meets statutory demands (Skal, Zwolle 2009). One of those statutory demands is that organic crops are grown in soil. But crop damage caused by nematodes is an important obstacle for profitable and sustainable cropping. Soil steaming and root stock are currently used as solution for the nematode problem. But soil steaming is very expensive and partially kills soil life; this is why many organic growers consider it as necessary but undesirable. Root stock currently only offers a partial solution because the soil usually contains several types of root knot nematodes against which no resistance is available while resistance can be broken by high population densities and high soil temperatures.

This report first deals with the nematode problem: What are nematodes and which nematodes are harmful in agricultural and horticultural crops (Chapter 1)? The problems for organic vegetable and flower cultivation are then described in Chapter 2. The current root knot nematode (*Meloidogyne* sp.) control options by soil steaming and rootstock are described in Chapter 3. The investigated control options (Chapter 4) are followed by a conclusion and discussion (Chapter 5).

The root knot nematode control options (Chapter 3) are interlarded with the results from this research. Details, such as experimental setup and testing method, are omitted to improve readability. The authors can be approached for information about the setup and further details on the experiments.

3 Current control options

3.1 Soil steaming

Soil steaming is currently the only effective method available to greenhouse growers for controlling plant parasitic nematodes. It is not preferred by growers but is a necessity to prevent large yield losses. In addition, soil steaming can result in a production increase, as has been documented for the cultivation of chrysanthemums in the first cultivation round after soil steaming (Van der Wurff 2009, unpublished data) by the release of, e.g., biologically available carbon. Hot steam is blown underneath a canvas cover and sometimes actively transported down wards into the soil by means of steam drainage. The costs, including costs of investment, are at least € 3.35 per square metre (in 2008), which makes it an expensive method (Box 3).

BOX 3 Soil steaming

Annual costs of steaming with steam drainage in 2008 (steaming once):

0.90 € / m^2 depreciation, labour (accurate work) and maintenance (incl. biannual inspection of € 6 000.-)
For organic cultivation labour and depreciation for steaming is estimated at 1.50 € / m^2 in view of the extra work for temporary removal of tube rail and sprinkler installation.
2.10 € / m^2 gas (7 m^3/m^2 on sandy soils, 5 m^3/m^2 on clay soils) – calculations at 30 ct / m^3

Total: 3.00 € / m^2 (3.60 € / m^2 in Eco-cultivation)

Best steaming results are obtained with soil steaming with underpressure, 'steam drainage'. This technique involves placement of a drainage net at 60 cm depth, which is used to suck steam deeper into the soil. A steam drainage system means an extra investment of 0.35 € /m^3, but could save 0.8 m^3/m^2 gas per treatment (Bloemisterij 22 November 2006) – interviewees estimate a possible saving of 2 m^3/m^2:

0.35 € / m^2 investment costs steam drainage
0.90 € / m^2 depreciation, labour and maintenance. For organic cultivation: 1.50 € / m^2
1.50 € / m^2 gas (5 m^3/m^2 on sandy soils, 3 m^3/m^2 on clay soils) – calculations at 30 ct / m^3

Total: 2.75 € / m^2 (3.35 € / m^2 in Eco-cultivation)

From: Vermeulen et al. 2008

Steaming is a generic measure against all harmful soil organisms, including *Verticillium* (killed at 60°C), *Pythium* (55°C) and *Fusarium* (72°C), root centipedes (60°C) and nematodes (50°C) (Sleegers 2008). But hot steam does not only kill harmful nematodes but also beneficial species (Chapter 1). It is unknown how long it takes before beneficial nematodes and other beneficial soil life returns. Roux-Michollet *et al.* (2008) report that, despite the fact that the total bacterial biomass recovers within 15 to 62 days after steaming (the so-called suppressiveness of a system; see Wurff van der *et al.* 2006), especially nitrifying bacteria (responsible for the transformation of ammonium into nitrate) had not yet returned within 62 days. This is because this group, contrary to e.g. denitrifiers (transformation from nitrate to ammonium) are poorly resisting high temperatures. The composition of the microbial soil community also changed considerably. Despite the fact that total bacterial activity (measured by SIR; oxygen transformation) and denitrification recovered rapidly, the values remained lower than in soils that were not steamed. The long-term effects of steaming on the nitrate cycle and other soil functions is unknown.

[3] Closed system with two tubes per 3.20 metre = 2.50 €/m2; assuming 7% depreciation, 1% maintenance and 6% investment costs = 35 ct/m^2

3.2 Rootstock

Organic cultivation of cucumber, sweet pepper as well as tomato faces root knot nematode problems (*Meloidogyne* spp.). The use of rootstock immune or tolerant to these nematodes is one of the means to restrict losses. Plants infested with root knot nematodes are often more sensitive to drought, lag behind in growth and production, and are more sensitive to other infestations such as *Verticillium* and corky root disease (*Pyrenochaeta* sp.; Hazendonk & Amsing 2002).

In the search for resistant rootstock against *Meloidogyne* spp. a distinction is made between tolerance and resistance. Resistance is the capacity of the host plant to restrict or prevent the growth and activity of plant-parasitic nematodes. Tolerance is the capacity of the host plant to show good growth and production despite nematode infestation of the roots. This means that tolerant rootstock reduces damage to the plant without tackling the cause of the damage. Rootstock without a certain degree of resistance does cause a strong increase in root nematodes in the soil (Hazendonk & Amsing 2002, Janse *et al*. 2007a,b). In due course this leads to great damage to the roots and thus production, and even plant loss, even if the rootstock is known to be tolerant.

In organic greenhouses, the occurrence of root knot nematodes is one of the most important reasons for significant yield losses in tomato *(Solanum lycopersicum* L.*)*, sweet pepper *(Capsicum annuum* L.*)* and cucumber *(Cucumis sativus* L.*)*. Results show that tested rootstocks of cucumber, tomato and sweet pepper differ greatly in resistance against root knot nematodes. Rootstocks with a relatively low susceptibility to root knots in cucumber were cvs. *64-10* and *Harry*. These cucumber rootstocks are more susceptible to *M.incognita* than to *M. hapla*. One of the best performing rootstock in tomato with a limited amount of root knots and a low reproduction of nematodes within the roots was cv. *PG76*. An interesting sweet pepper rootstock for organic growing is cv. *Snooker*. Most of the tested rootstocks in tomato and sweet peppers have a higher resistance against *M.incognita* compared to *M. hapla.*

3.2.1 Cucumber

Of the three fruit vegetable crops, organic cucumber cultivation is by far most susceptible to *Meloidogyne* infestation (LaBrie 2009). Damage may rise to 30% production loss. Measurable damage occurs even at a slight visual infestation. Especially *Meloidogyne incognita* causes the most serious problems in organic cucumber cultivation. *M. javanica, M. hapla* and *M. arenaria* are important as well.

Table 7. Average root-knot index (RKI) and root weight (RW) in grams for cucumber rootstocks in five experiments.

Cultivar	Parental species	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5	
		RKI	RW†	RKI	RW†	RKI	RW†	RKI	RW†	RKI	RW†
Aviance[1]	*Cucumis sativus*	8.1^{e}	202^{bc}	7.3^{c}	82^{b}						
Azman[1]	*Cucurbita maxima x C. moschata*	7.6^{e}	244^{bc}								
Harry[3]	*Sycios angulatus*	3.5^{b}	331^{c}	2.4^{a}	110^{b}	3.3^{ab}	84	3.6	4	2.5^{a}	42^{ab}
TZ148[4]	*Cucurbita maxima x C. moschata*			6.1^{b}	67^{a}						
RZ81-07[1]	unknown	6.6^{de}	199^{bc}								
RZ82-07[1]	unknown	5.8^{cd}	157^{ab}								
RZ64-10[1]	*Benincasa*	2.0^{a}	93^{a}	3.2^{a}	83^{b}	2.4^{a}	66	3.6	8	2.4^{a}	37^{a}
RZ64-12[1]	*Benincasa*	5.3^{c}	137^{ab}	2.9^{a}	76^{b}	3.8^{abc}	58	3.9	12	3.3^{b}	48^{b}
Adrian[1]	*Cucumis sativus*	7.4^{e}	202^{bc}								
E88.035[2]	*Cucurbita maxima x C. moschata*			7.3^{c}	56^{a}						
E88.036[2]	*Cucurbita maxima x C. moschata*			6.2^{b}	66^{a}						
WS5299[5]	*Cucurbita maxima x C. moschata*			6.2^{b}	87^{b}	6.6^{c}	53				
08-29[1]	unknown					4.8^{abc}	52				
08-53[1]	unknown					5.7^{bc}	33				
Becada[1]	unknown					6.2^{c}	47				
Sakata Kohai Fushinari[6]	*Cucumis sativus*					5.8^{bc}	37				

Root knot index (RKI) ranges from 0 to 10, where 0 represents an absence of root-knots.
Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.
†RW= root weight (grams) [1]Rijk Zwaan, [2]Enza seeds, [3]Syngenta, [4]Clause, [5]Uniseeds, [6]Centre for Genetic Resources, The Netherlands.

It is difficult to ascribe crop damage fully to *Meloidogyne*; secondary damage as result of the weakened crop (e.g. fungus infestation, which in turn causes crop damage) often occurs. A recent literature study (Labrie 2008) showed that rootstock resistant to *M. incognita* does not yet exist for cucumber. There is rootstock with a high degree of tolerance resulting in fewer knots and eggs being formed. The rootstock *Sycios angulatus* 'Harry', frequently used in cucumber, is highly tolerant to *M. incognita* (Table 7 and 8; Janse *et al.* 2007a, b). Grafting of plants on rootstock involves extra costs for plant material and may give 10-20% production loss. A review of tested cucumber rootstock is presented in Tables 7 and 8.

From 2005 to 2009, five experiments were conducted. In experiments 1 and 2, rootstocks with cv. *Aviance* were compared with ungrafted cv. *Aviance*. In experiments 3 and 4, rootstocks were not grafted. In experiment 5, cucumber rootstocks were grafted with the powdery mildew resistant cvs. *Sudica* and *Shakira* (Monsanto) and cv. *Aviance* (Rijk Zwaan).

Experiment 1 to 5 started respectively in March 2007 and extended fourteen weeks with ten replicates per rootstock; August 2007 for eleven weeks with eight replicates per rootstock, March 2008 for twelve weeks with five replicates per rootstock, August 2008 for ten weeks with five replicates per rootstock and March 2009 for twelve weeks with nine replicates per rootstock. For experiments 1 to 3, $16*10^3$, $9.3*10^3$, $4*10^3$ *M. icognita* was inoculated per pot, respectively. In experiments 4 and 5, mixed populations of $19.2*10^3$ with *M. incognita* : *M. hapla* of 1:1 and $20.3*10^3$ with *M. incognita* : *M. hapla* : *M. javanica* of 64:33:3 were used.

All experiments were performed in a greenhouse of $144m^2$ at Bleiswijk, The Netherlands, with temperatures ranging from 21.6 to 22.2°C. The minimum temperature was 18°C during the night and maximum temperature was 30°C during the day.

Rootstocks were organically propagated and rooted. After approximately 40 days, plantlets were transferred to ten liter pots. Pots contained coarse sand, were sealed with agryl cloth and positioned in rows with about two plants per m^2. Pots were irrigated with a standard nutrient solution with an EC of 1.7 mS/cm. Stem diameters of plants were measured at a height of 15 cm above soil.

From two experiments, namely experiment 4 and 5 (Table 7, 8), it can be concluded that cvs. *Harry, RZ64-10* and *RZ64-12* were more susceptible to *M. incognita* compared to *M. hapla* or *M. javanica* (Table 2). Seed production of *RZ64-10* and *RZ64-12 (Benincasa* sp.*)* was discontinued. At this moment, cv. *Harry* (*Sycios angulatus*) seems to be the only suitable and available rootstock for organic growers. However, cv. *Harry* has some disadvantages, namely variability in germination, moderate compatibility with scion, susceptibility to rot at the grafting place during growth and, despite the less visible root-knots, ongoing reproduction of root knot nematodes and thus an increase in *Meloidogyne* population size in soil.

Table 8. *Average number of root-knot nematodes (RKN) per 50 gram roots in cucumber rootstocks in five experiments.*

Cultivar	Exp. 1	Exp. 2	Exp. 3	Exp. 4*		Exp. 5*		
	Mi	*Mi*	*Mi*	*Mi*	*Mh*	*Mi*	*Mh*	*Mj*
Aviance	414500[b]	41750[b]						
Azman	459500[b]							
Harry	415500[b]	21125[b]	100561[abc]	25283	225	71	2	0
TZ148		19125[b]						
RZ81-07	488500[b]							
RZ82-07	435000[b]							
RZ64-10	34000[a]	22500[b]	24485[a]	2235	63	121	0	0
RZ64-12	58500[a]	35625[b]	137639[abc]	2060	12	240	8	0
Adrian	276500[b]							
E88.035		14250[a]						
E88.036		15500[a]						
WS5299		35625[b]	601550[c]					
08-29			100639[abc]					
08-53			655907[c]					
Becada			266062[bc]					
Sakata Kohai								
Fushinari			22646[ab]					

Mi = Meloidogyne incognita, Mh = M. hapla, Mj = M. javanica.
Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.
**Numbers are estimated based on total Meloidogyne counts and Mi, Mh and Mj specific quantitative PCR markers.*

3.2.2 Sweet pepper

Root knot nematode damage also occurs in sweet pepper. Contrary to cucumber, however, resistant rootstock are available. This resistance, however, is not complete and does not hold for all *Meloidogyne* species. Rootstocks may be resistant to *M. javanica* but complete resistance to *M. incognita* does not exist (Labrie 2008). Crop damage in sweet pepper is relatively small (<5%) (R. Berkelmans, pers. comm. 2008). Because *Meloidogyne* shows continued growth on sweet pepper, a crop grown after sweet pepper, however, will be facing a high nematode pressure. This means that (high) yield losses in succeeding crops are possible.

Table 9. Average root-knot index (RKI) for sweet pepper rootstocks in five experiments.

Cultivar	Exp. 3	Exp. 4	Exp. 5
Capital	0.0	1.3^{ab}	0.6^{a}
3412	0.2	2.0^{abc}	5.0^{d}
3413	0.0	3.0^{bc}	4.8^{d}
Snooker	0.0	3.2^{bc}	3.0^{bcd}
PR131	0.0	1.8^{abc}	0.4^{a}
PR138	0.0		
PR147	0.0		
PR156	0.0	3.2^{bc}	3.4^{cd}
Brutus	0.0	3.4^{c}	
WS2004	0.0	2.5^{abc}	
E43.1232	0.0		
E43.9587	0.0		
07zs102		1.0^{a}	1.0^{ab}
E43.2213		3.0^{bc}	
E43.2217		2.5^{abc}	
E43.2262		2.4^{abc}	
Ferrari		3.0^{bc}	2.2^{abc}

Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.

In experiment 3, almost no root knots were found in all tested sweet pepper rootstocks (Table 9). In the next two experiments, *Capital* and *07zs102* performed relatively well in terms of RKI (Table 9). However, *07zs102* had high reproduction of especially *M. hapla* in experiment 5 (Table 10).

Table 10. Average number of root-knot nematodes (RKN) per 50 gram roots in sweet pepper rootstocks in three experiments.

Cultivar	Exp. 3	Exp. 4		Exp. 5		
	Mi	*Mi*	*Mh*	*Mi*	*Mh*	*Mj*
Capital[3]	11200	41	4245	62	4993	0
3412[3]	4948	5	3333	16	3630	0
3413[3]	2420	8	2945	34	3582	0
Snooker[4]	8703	8	2771	23	3020	0
PR 131[2]	6043	0	1489	23	2515	0
PR 138[2]	10815					
PR 147[2]	15885					
PR 156[2]	3770	0	5957	107	6557	0
Brutus[5]	13186	0	16324			
WS2004[3]	18911	0	15738			
E43.1232[1]	12024					
E43.9587[1]	4642					
07zs102[6]		0	3301	30	11089	0
E43.2213[1]		0	11682			
E43.2217[1]		11948	17052			
E43.2262[1]		1487	29224			
Ferrari[1]		1258	4892	11085	2032	0

Mi = Meloidogyne incognita, Mh = M. hapla, Mj = M. javanica.
Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant (Exp. 3) or roots were pooled (Exp. 4, 5).
[1]Enza Seeds, [2]Rijk Zwaan, [3]Monsanto, [4]Syngenta, [5]Gautier seeds, [6]Uniseed.

All tested rootstocks in experiment 4 and 5, except *E 43.2217* and to a lesser extent *E 43.2262*, showed a much higher resistance against *M. incognita* compared to *M. hapla*. Although only 3% of the inoculated nematodes were *Mj*, the tested rootstocks seemed to have some resistance against *M. javanica* since no *M. javanica* were extracted from roots.

The standard cv. *Ferrari* proved to be susceptible to *M. incognita.* Especially in the last experiment, reproduction of *M. incognita* in cv. *Ferrari* was very high (Table 10).

Of all rootstocks, cv. *3413* had the highest root weight (Table 11) and it may be expected that this rootstock has a strong growing power (stem diameter is 14.5 mm; Table 11). Cvs. *Capital*, *WS2004*, *PR 131* and *Ferrari* had the largest stem diameter (Table 11).

Table 11. Average root weight (RW) in grams and stem diameter (mm) of sweet pepper rootstocks in the experiments.

Cultivar	RW†			Stem diameter (mm)		
	Exp. 3	Exp. 4	Exp. 5	Exp. 3	Exp. 4	Exp. 5
Capital	23	18	41^{bc}		13.6^{ef}	17.5^{b}
3412	49	18	65^{cd}		10.8^{abc}	14.7^{a}
3413	57	26	83^{d}		10.7^{abc}	14.7^{a}
Snooker	33	21	56^{cd}		10.4^{ab}	15.2^{ab}
PR131	24	24	26^{ab}		14.5^{f}	16.1^{ab}
PR138	14					
PR147	22					
PR156	40	24	42^{bc}		12.4^{cde}	16.1^{ab}
Brutus	30	19			9.8^{a}	
WS2004	36	16			13.2^{ef}	
E43.1232	29					
E43.9587	25					
07zs102		10	23^{ab}		11.9^{cde}	14.7^{a}
E43.2213		18			11.7^{bcd}	
E43.2217		12			12.8^{def}	
E43.2262		15			13.2^{def}	
Ferrari		6	19^{a}		13.6^{ef}	16.9^{ab}

Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.
RW† =root weight (grams).

3.2.3 Tomato

With an estimated yield depression of 10%, damage in tomato as a result of root knot nematodes, is relatively small. Tomato can be considered a strong crop with a strong root system, usually keeping up under less favourable conditions. Secondary damage, however, probably does occur but this is difficult to quantify. In practice, e.g., a high white fly or *Verticillium* infestation is observed at times of high nematode populations. Yield depression does at the moment certainly occur but it is unknown whether this is the result of the nematodes or of the secondary infestation (white fly cq. *Verticillium*). And to what extent can the occurrence of white fly or *Verticillium* be attributed to the presence of nematodes in the soil. Estimation of crop damage as result of *Meloidogyne* is difficult in view of these various cause-effect relationships.

In experiment 3, all rootstocks showed a low root knot index (RKI), except for cvs. *Vigostar 4411* and *E28.33458.* In addition, both showed high RKN reproduction rates (Table 12) of *M. incognita*.

Experiment 4 confirmed that the best rootstocks in experiment 3 have resistance against *M. incognita*, because they showed a low root knot index and reproduction of *M. incognita* compared to the standard cv. *Mecano*. Cv. *Vigostar 4409* showed a low reproduction of *M. hapla* as well as of *M. incognita*. Unfortunately, seed production of *Vigostar 4409* was discontinued, therefore it was not investigated further. Nearly all rootstocks have a higher reproduction rate of *M. hapla* than of *M. incognita*, with on average a factor eight difference (Table 12).

In experiment 5, especially cv. *PG76* showed a low root knot index. In contrast, all rootstocks of Green Seeds appeared to have little or no resistance against *M. incognita*, *M. hapla* or *M. javanica.* Despite the fact that plants were inoculated with twice as much *M. incognita* compared to *M. hapla*, reproduction of *M. hapla* was, with exception of the varieties of Green Seeds, on average ten times higher. A similar result was obtained in experiment 4. Remarkably, the average root knot index in experiment 5 was relatively high compared to the two previous experiments; however, the number of offspring was lower when compared to experiment 3.

Of the six rootstocks investigated in all three experiments, cv. *Maxifort* and cv. *Emperador* appeared to have high-, and cvs. *DR0132* and *PG76* seemed to have relatively low reproduction of *Meloidogyne spp.*

PG76 and to a lesser extent cv. *Brigéor,* showed a low root knot index. Both tomato rootstocks performed well. Especially cv. *PG76* seems promising since it combined a low root knot index with low reproduction. Similar results were obtained by Cortada *et al.* (2009).

Standard cv. *Mecano* and rootstocks of Green Seeds had the thinnest stems and were therefore expected to grow less fast (Table 13). Generally, it is assumed that root weight and stem thickness are indicators of growth rate.

Table 12. Average root-knot index (RKI) and number of root-knot nematodes (RKN) per 50 gram roots in tomato rootstocks in three experiments.

Cultivar	RKI			RKN					
	Exp. 3	Exp. 4	Exp. 5	Exp. 3	Exp. 4		Exp. 5*		
					Mi	*Mh*	*Mi*	*Mh*	*Mj*
Maxifort[1]	0.6	0.8^{ab}	5.3^{cdef}	40246^{abc}	1707	993	137	716	0
Multifort[1]	0.6			4000^{a}					
Optifort[1]	0	1.1^{ab}	4.4^{cde}	2330^{a}	714	4201	27	333	0
DRO132[1]	0	1.8^{ab}	4.2^{cde}	2770^{a}	92	1319	8	225	0
DRO136[1]	0			2337^{a}					
Resistar[9]	0	1.5^{b}		5181^{a}	97	2064			
Integro[10]	0			10060^{abc}					
Vigostar 4409[6]	0	0.9^{ab}		25371^{abc}	150	282			
Vigostar 4411[6]	6.0			141201^{bc}					
RS7122[2]	0			31410^{abc}					
RS7123[2]	0	0.8^{a}		23520^{abc}	225	5655			
Emperador[2]	0	1.4^{ab}	3.4^{bc}	3570^{a}	681	4775	77	799	4
Brigéor[7]	0	1.0^{ab}	1.9^{ab}	9292^{abc}	295	5162	23	841	2
PG76[7]	0	0.3^{a}	0.6^{a}	6540^{ab}	172	1814	31	127	0
Titron[1]	0			5509^{ab}					
E28.33197[4]	0			20327^{abc}					
E28.33458[4]	5.2			990760^{c}					
E28.33464[4]	0	0.6^{b}	3.6^{bcd}	22452^{abc}	36	4125	24	243	0
500267[5]	0.2			7710^{abc}					
500294[5]	0.2	1.1^{ab}		5230^{ab}	3	3717			
ST3505[8]	0								
Big Power[2]			2.1^{ab}				34	457	0
No5[3]			6.0^{ef}				5434	1732	189
No7[3]			4.8^{cdef}				3855	2061	155
AN-67[3]			5.0^{def}				4416	4526	156
Tyking 5[3]			4.2^{cdef}				2771	1455	262
DRO138[1]			4.7^{cdef}				103	935	0
Mecano[2]		5.3^{d}	6.4^{f}		5013	883	4977	819	0

Mi = Meloidogyne incognita, Mh = M. hapla, Mj = M. javanica.
Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.
** Numbers are estimated based on total Meloidogyne counts and Mi, Mh and Mj specific quantitative PCR markers.*
[1] Monsanto, [2] Rijk Zwaan, [3]Green Seeds, [4]Enza seeds, [5]Syngenta, [6]Nickerson-Zwaan, [7]Gautier seeds, [8]Uniseed, [9]Hazera, [10]Western Seed.

Table 13. Average root weight (RW) in grams and stem diameter (mm) of tomato rootstocks in the experiments.

	RW† (g)			Stem diameter (mm)	
Cultivar	Exp. 3	Exp. 4*	Exp. 5	Exp. 4	Exp. 5
Maxifort	80^{abcd}	28	150^{d}	13.7^{bc}	19.5^{de}
Multifort	107^{bcd}				
Optifort	138^{bcd}	25	93^{cd}	13.2^{abc}	18.4^{de}
DRO131	113^{bcd}	15	119^{d}	14.4^{bc}	17.9^{de}
DRO132	76^{abcd}				
Resistar	47^{ab}	29		13.7^{bc}	
Integro	126^{bcd}				
Vigostar4409	32^{ab}	25		12.7a^{b}	
Vigostar4411	40^{abc}				
RS7122	89^{bcd}				
RS7123	156^{bcd}	15		14.0^{bc}	
Emperador	133^{bcd}	29	75^{cd}	14.2^{c}	18.3^{de}
Brigéor	142^{bcd}	29	68^{abc}	13.5^{bc}	14.4^{cde}
PG76	150^{cd}	19	69^{bcd}	13.7^{bc}	16.4^{cde}
Titron	91^{abcd}				
E28.33197	42^{abc}				
E28.33458	219^{d}				
E28.33464	34^{a}	36	110^{d}	11.2^{a}	16.4^{cde}
500267	228^{d}				
500294	174^{cd}	24		13.1^{ab}	
ST3505	64^{abcd}				
Big Power			87^{cd}		15.9^{bcd}
No5			46^{abc}		13.8^{abc}
No7			32^{a}		13.4^{ab}
AN-67			27^{a}		12.4^{a}
Tyking5			30^{a}		13.5^{abc}
DRO138			124^{d}		19.7^{e}
Mecano		26	35^{ab}	12.8^{abc}	13.2^{a}

Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data. If letters are not indicated, the F-test was not significant.

†RW = root weight.

**Roots were pooled.*

4 New options for control?

4.1 Soil amendments, plant strengtheners and Plant protection products of Natural Origin (PNOs)

Research has in recent years hardly yielded natural or non-living products with a controlling effect on root knot nematodes. This means that biological control will have to be integrated with other measures as described in the Conclusion and discussion of this report (Chapter 5). This Chapter presents the tested soil amendments, plant strengtheners and PNOs. PNOs are Plant protection products of Natural Origin. A review of the substances and methods mentioned here, with an effect on root knot nematodes, is presented in Annex 2. The PNOs were tested in laboratory experiments (such as § 4.3 Phytochemicals), pot experiments and field experiments. The effect of the PNOs on numbers of free-living root knot nematodes (J2) in the soil, size and amount of root knots (root knot index, RKI, Annex I) and number of progeny were investigated. The last test involved 'luring' of the progeny out of the roots in a so called mistifier.

Manual counting of root knot nematodes under a binocular was the mainly used method in these experiments. But this overlooks species-specific effects of PNOs on root knot nematodes in field experiments (§4.4.1). A DNA detection technique (§4.4.2; for further details see §1.4) was used to still determine the effect of products on species in field experiments.

4.1.1 Effects on root knot nematodes *sensu lato*

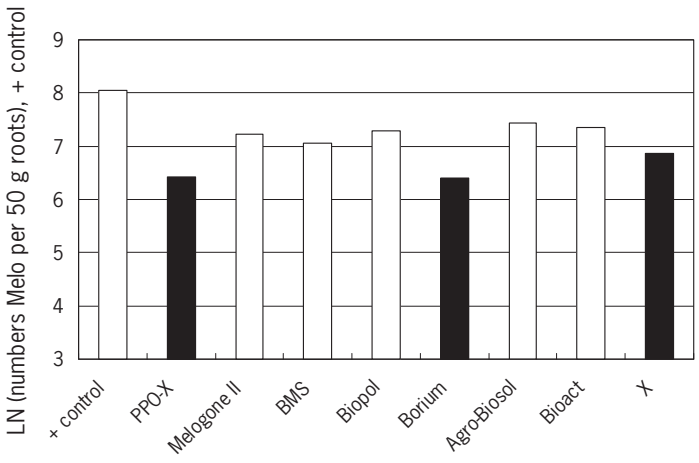


Figure 3. *Effectiveness of products or plant strengtheners against M. incognita in pot experiments in Bleiswijk. Products and 10 000 root knot nematodes (M. incognita), respectively, were applied to 10-l pots. A tomato plant (cv. Mecano) was then planted after 7 days. For each plant, the amount of nematodes per 50 g roots was counted after termination of the experiment (20 weeks). Each treatment consisted of 7 plants, arranged in a so-called block-design with 4 blocks. Products/plant strengtheners were plant extract PPO-X; Melogone II (DCM), product X (BMS), Biopol product X, borium, Agro-Biosol and product X (Koppert), respectively.*

Figure 3 shows the average logarithm of the number of nematodes per 50 g roots. The experiment was terminated 20 weeks after planting and root systems and visual infestation were rated by using the Root Knot Index (RKI, see Annex 1). The nematodes originating from the harvested roots were counted four weeks after termination of the greenhouse experiment. The black columns are significantly different from the positive control. Plant extract PPO-X

belongs, together with borium and an unauthorised of Koppert (X), to the significantly effective products. An effect of borium has been described before by Castro *et al.* (1990). The mechanism, however, is not clear. Excess borium, however, may also cause serious crop damage and larger knots (Berkelmans 2009 unpubl. data). In 2008 the soil amendment caliente (PHC) was tested in four plots on a commercial holding. The product was irrigated down and the soil was then covered. Four untreated plots were analysed as well as controls. The numbers of nematodes in the soil were analysed quantitatively with a DNA test (qPCR on *M. incognita*). The difference between the treated and untreated plots was only seen in greenhouse MK (Figure 4) because infestation in greenhouse DM was low. This only concerns the analysis of a mixed sample; Figure 4 can therefore only be seen as an indication.

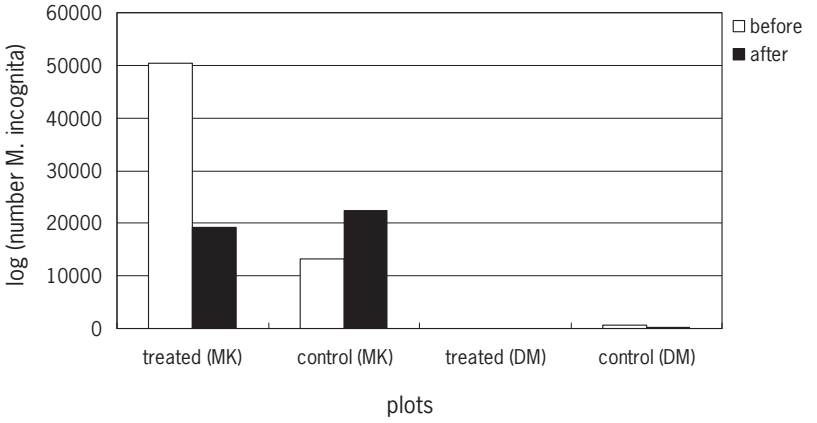
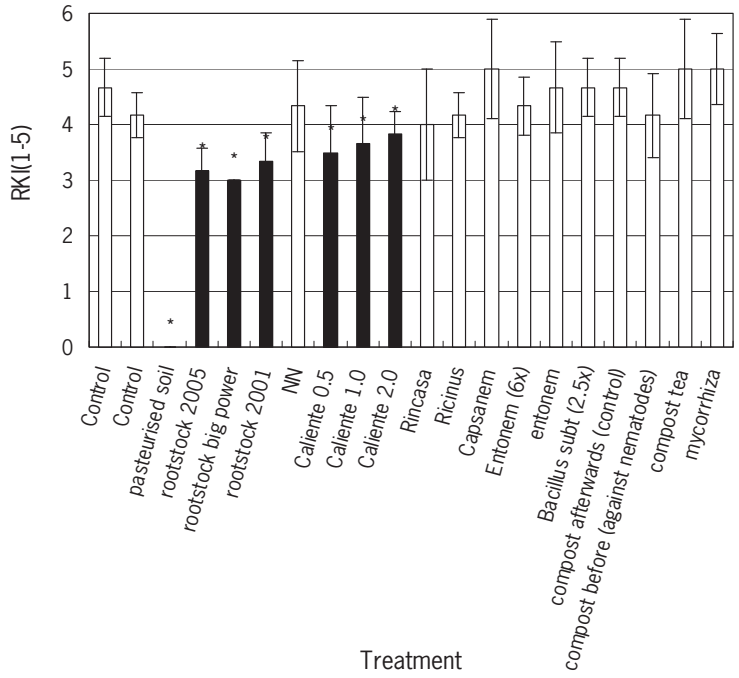
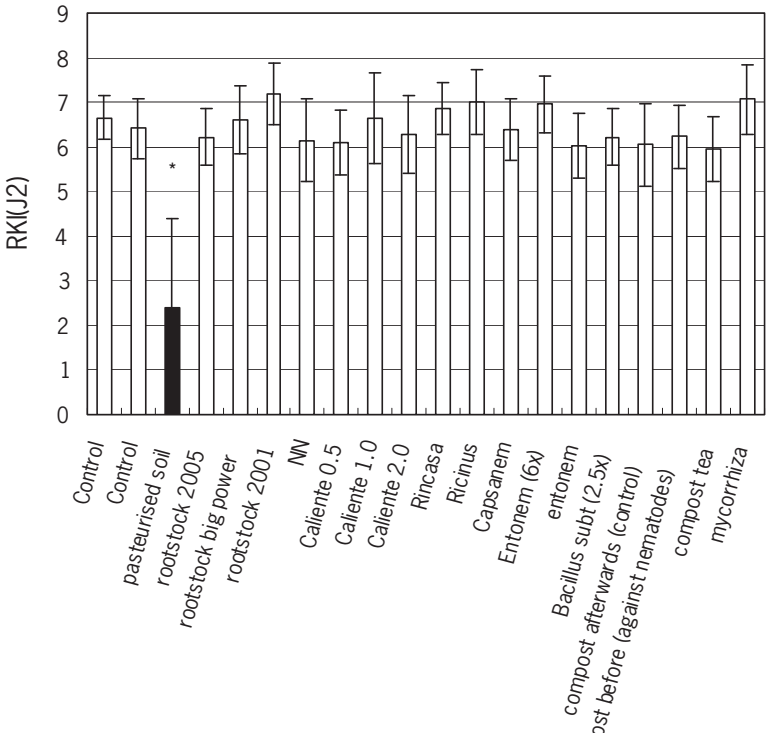


Figure 4. *Effect of a liquid caliente (PHC) treatment in a field experiment in two greenhouses (MK and DM).* ***The*** *graph shows a difference between the number of (J2) root knot nematodes in the caliente treatment and the untreated plots (control) on a commercial holding in greenhouse MK. The product was irrigated down and covered. Treatments comprised four plots. A mixed sample with soil from the four plots was used to determine the number of M. incognita with a species-specific qPCR DNA test.*

In the pot experiment caliente (PHC) also reduced visible root damage as scored by the numbers of root knots (Figure 5a). But he product had no visible effect on the number of progeny in the roots (Figure 5b). In this test the effect of rootstock on root damage was larger than the effect of the various biological products (Figure 5a). The number of progeny from the roots of the rootstock used was neither significantly different from the untreated controls.



A.



B.

Figure 5. *A. Effectiveness of biological products in a pot experiment on the number of root knots. The graph shows that rootstock 2001, 2005 and Big Power (Rijk Zwaan) and an unauthorised liquid product named Caliente give a reduction of the number of root knots as scored with the Root Knot Index (RKI). B. Effectiveness of biological products in a pot experiment on the number of juveniles in egg masses within the roots. The graph shows that only pasteurisation of the soil gives a reduction of the number of progeny (J2) scored as the number of J2 leaving the roots after 28 days incubation in the misting room (luring test). Significant differences with the untreated control are marked with an *. Each treatment consisted of 7 pots, arranged in a so-called block design with 4 blocks.*

In a field experiment the unauthorised product rincasa (PRI; incl. mustard) was tested with the plant-strengthener nutrineme, biofumigation with yellow mustard, and caliente (PHC) (Figure 6). Here nutrineme showed no effect but biofumigation (covered with foil) and rincasa (PRI) decreased root damage.

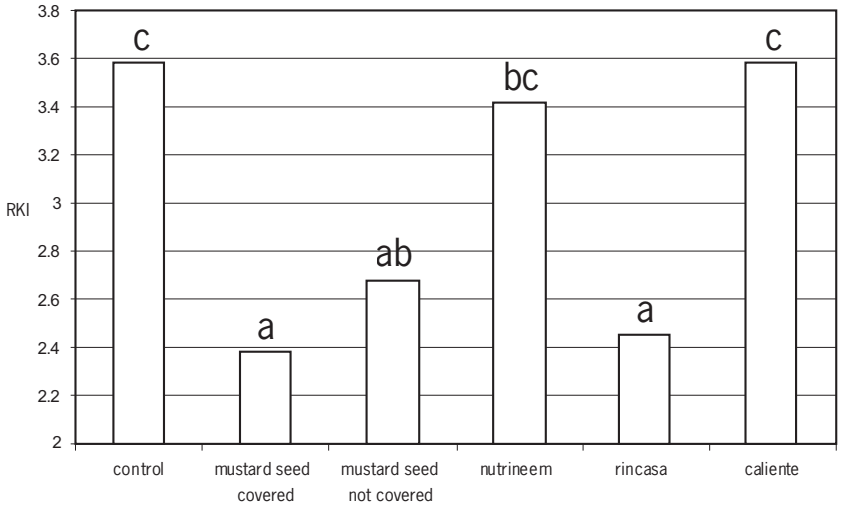
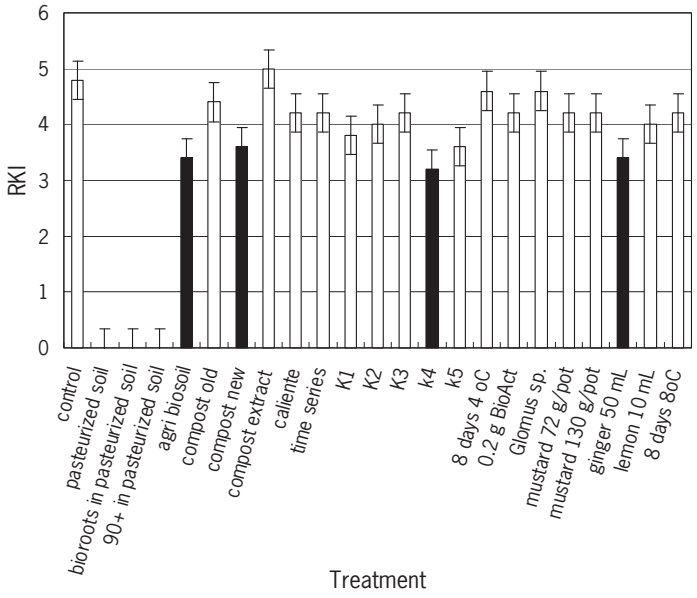
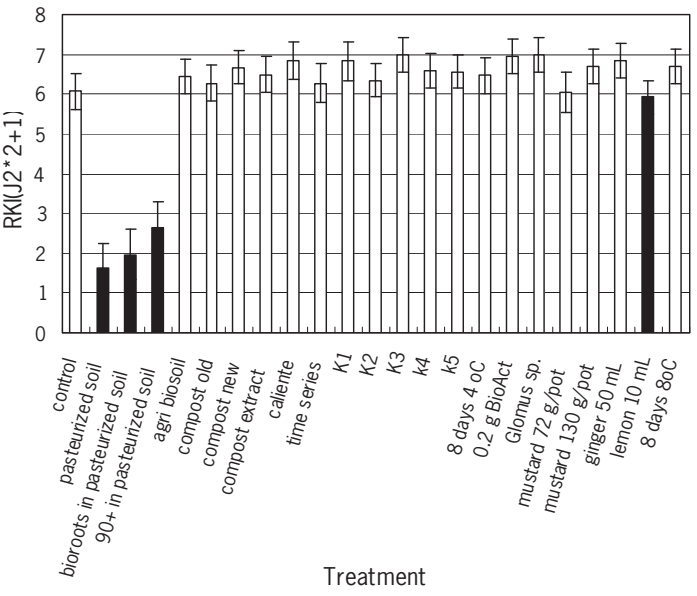


Figure 6. *Effectiveness of biological products in a field experiment on the number of root knots. Biofumigation with yellow mustard (covered) and the unauthorised product rincasa (PRI) and to a lesser extent biofumigation (not covered) and the plant strengthener nutrineme (NTS) show a decrease in the number of root knots (RKI). Treatments were set up in three blocks with two replicates within a block. Significantly different groups are marked by different letters (a, b, c).*

Rincasa was then tested in a pot experiment in which the effectiveness of five different compositions was compared (K1-5; Figure 7). Caliente (PHC) was again included in the experiment. Only the rincasa K4 variant showed an effect but the RKI was only 2 points lower than the untreated control (no steaming) (Figure 7a). The treatments with Agro Biosol, a compost tea with ginger oil (50 ml per 10-l pot) also showed a decrease in the amount of root knots. The treatments with lower temperatures, such as 8 days at 8 and 4 °C and the different types of compost, BioAct, dried mustard granules, and the commonly occurring mycorrhiza *Glomus* sp. showed no effect on the number of root knot nematodes. Only the treatment with lemon grass oil reduced the number of progeny (Figure 7b). This may be caused by a so-called 'delayed resistance reaction', where root knot nematodes penetrate the roots and cause root knots, but the number of progeny is reduced by a resistance reaction of the plant. But nematodes were still found in the control pots that had not been infested with nematodes (Figure 7b). This can be explained by an infestation with root knot nematodes of the root samples in the mistifier. This sheds doubt on the results in Figure 7b. If it would nevertheless be found that systematically fewer nematodes would be found in several samples of a treatment, as in the above-mentioned treatment with lemon grass oil, it can be concluded that there has been a strong effect.



A.



B.

Figure 7. *A. Effectiveness of biological products in a pot experiment on the number of root knots. Black bars differ significantly from the unsteamed control. The graph shows that Agri Biosol, a compost type, K4 (Rincasa variant) and ginger give a reduction in the number of root knots scored as RKI.*
*B. Effectiveness of biological products in a pot experiment on the number of juveniles in the egg mass within the roots. The graph shows that only pasteurisation of the soil reduces the number of progeny (J2) scored as the number of J2 leaving the roots after 28 days incubation in the misting room (luring experiment). Significant differences with the untreated control are marked with an *. Each treatment consisted of seven 10-l pots, arranged in a so-called block design with 4 blocks.*

4.1.2 DNA detection: Species-specific effects

In the years until 2008 the effectiveness of products was determined by microscopic counts of nematodes in soil after washing (Oostenbrink funnel) and incubation for 28 days and washing (number of progeny from egg masses). This method made no distinction between different types of root knot nematodes. Molecular species identification enables counting at species level.

Molecular qPCR detection of the species *M. chitwoodi; M. fallax; M. minor; M. naasi; M. hapla; M. javanica; M. incognita; Melo* spp. (= unidentified root knot nematodes) was used to study the species-specific effects of products in a field experiment in 2008 with organic tomatoes. Four products were applied every two weeks throughout the year. In November 2008 three mixed samples were taken from a bed as well as three mixed samples (taken in pairs) from the adjacent and untreated bed as control. The following products were used: 1 = Prospernema; 2 = Agro Biosol 10 cc; 3 = Agro Biosol 20 cc; 4 = BioAct. Product 1 and 4 have no authorisation as pesticide and Agro Biosol is a fertiliser (plant strenghtener).

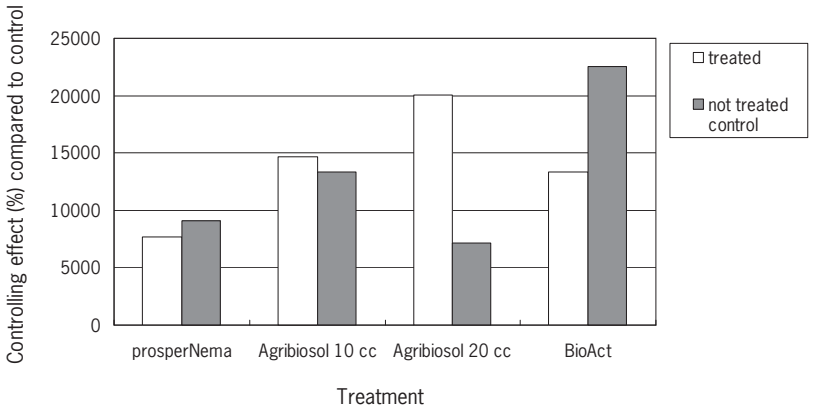


Figure 8. *Controlling effect of four biological products on the root knot nematode M. incognita. Only treatment 'Agri Biosol 20 cc' shows a significant difference between treated and untreated control; this treatment does not give a decrease but an increase in the number of nematodes on this holding.*

Figure 8 shows that treatment with a 20 cc dose Agri Biosol (a slow-release organic fertiliser) results in an increase in the root knot nematode *M. incognita*. According to the manufacturer Biosol contains 80% OM, 6-8% fixed N, 0.5% water-soluble N, 0.5-1.5% P_2O_5, 0.5-1.5% K_2O with a C/N-ratio 6:1. The number of root knot nematodes may possibly be increased by an increase in the number of root tips after application of this organic fertiliser. Root tips in particular are known for their luring effect on free-living juveniles of root knot nematodes (Chapter 1).

The other products showed no significant increase or decrease. The product BioAct, with the fungus *Paecilomyces lilacinus*, seems to be effective against *M. fallax* but not against *M. incognita*. No *M. fallax* is found in 2 of the 3 mixed samples of the treated plots whereas this nematode was found in 2 of the 3 untreated control plots sampled in pairs. The nematodes *M. chitwoodi, M. naasi* and *M. hapla* are found in very low numbers, ranging from 1 to 2 individuals per sample; this means that no reliable conclusions can be drawn for these species.

4.2 Phytochemicals

In Asia, plant extracts are traditionally frequently used to control nematodes. Extracts of the Neem tree are a well-known example of products with a long tradition, which some consider as the symbol of biocultivation.

Much research has been conducted into substances of vegetable origin with a nematicidal effect and a number of these are equally effective as chemical products. And some of these substances are at the same time increasing crop resilience. These natural substances can be applied in different ways. Application in the form of an extract is the best known method.

Use of crop residues is a hardly investigated method. Residues of sweet pepper and tomato were found to have some effect against root knot nematodes where it is reported in the literature that in particular the primitive strains show effectiveness by, e.g., inducing resistance of the plant. Another example mentioned in the literature is the controlling effect of dried and cut pieces of cucumber of *Cucumis myriocarpus* on *M. incognita* (Mashela 2007).

Worldwide research into substances of vegetable origin that may serve as replacement of synthetic pesticides has been going on for quite some time. The literature describes an enormous number of options (Table 14). Examples of natural products with an effect on root knot nematodes can be classified according to functional groups such as phenylpropanoids (simple phenols and flavenoids), polyphenols, (mono)terpenoids and alkaloids. These substances cause lethargy, activation, mortality, or freezing of the life cycle, e.g., by halting the egg stage (Wuyts *et al.* 2006).

Table 14. Examples of plants with known antagonistic effect against various soil pests and diseases.

Name	Scientific name
Coriander	*Coriandrum sativum*
Chrysanthemum	*Chrysanthemum marifolium*
Blanket flower	*Gaillardia grandiflora*
Sneezeweed	*Helenium spp*.
Marigold	*Tagetes spp*
Spinach	*Spinacia oleracea*
Henna	*Lawsonia inermis*
Blackeyed susan	*Rudbeckia hirta*
Thistle	*Cirsium japonicum*
Common rue	*Ruta graveolens*
Greater plantain	*Plantago major*
Japanese iris	*Iris japonica*
Wall rocket	*Diplotaxis virgata*
Topped lavender	*Lavendula stoechas*
Black cumin	*Bunium persicum*
Green onion	*Allium fistulosum*

In 2007 and in 2008 a number of plant extracts have been tested in the laboratory for survival of root knot nematodes (*M. incognita*; J2 stage) using a toxicity test in which extract was added to the nematodes in water. Survival was scored after 2 days. Plants were harvested during the flowering period and frozen for later analysis. Products PPO V, W, X, Y and Z (Figures 9 and 10) show an almost 100% mortality or lethargy. It is worth noting that an extract of above-ground parts of *Tagetes* 'ground control' have no killing or lethargic effect (Figure 10) whereas the roots do have such effects (Figure 9). Perspectives of these products as regards legal authorisation and effectiveness in field experiments will be investigated in follow-up research.

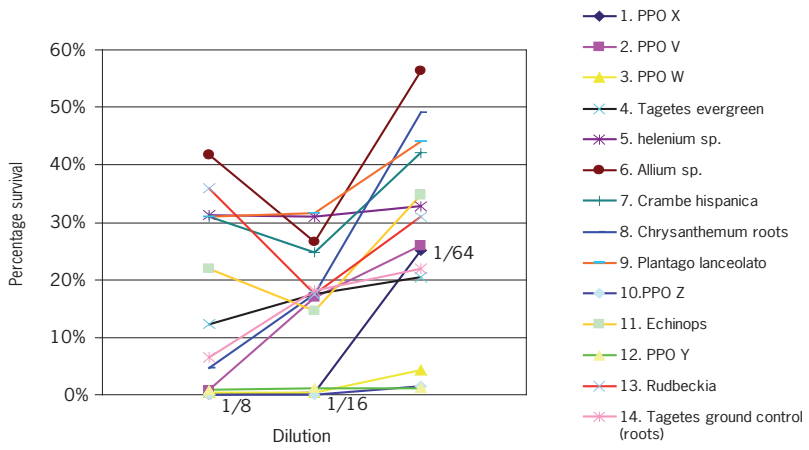


Figure 9. *Survival of M. incognita juveniles (J2) in comparison with the control (water) after 48 h incubation in plant extracts. The horizontal axis shows the dilution, 1/8, 1/16 and 1/64 dilution, respectively, of 30 g fresh weight plant material. The test makes no distinction between mortality and lethargy.*

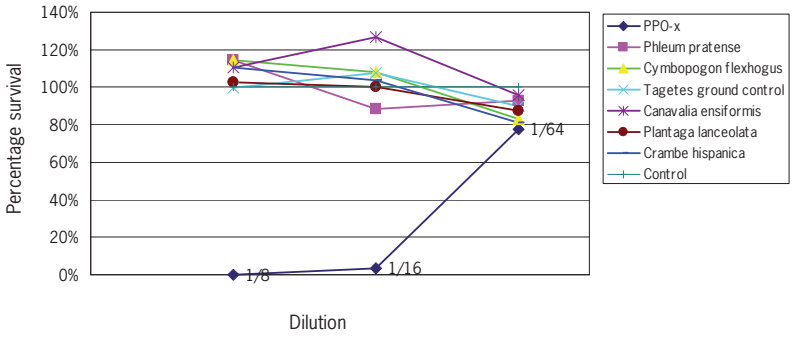


Figure 10. *Survival of M. incognita juveniles (J2) in comparison with the control (water) after 48 h incubation in plant extracts. The horizontal axis shows the dilution, 1/8, 1/16 and 1/64 dilution, respectively, of 30 g fresh weight plant material. The test makes no distinction between mortality and lethargy.*

4.3 Trap crops or antagonistic crops

Trap crops are plants in which nematodes penetrate without reproducing or plants that are removed before the end of the life cycle of root knot nematodes, i.e., formation of the J2 stage. Marigold (*Tagetes patula*) is a good example of a trap crop against root lesion nematodes (*Pratylenchus*). A cultivation of three months strongly reduces the number of root lesion nematodes. Crops that are in principle good host plants can sometimes also be used as trap crop. It is important that such trap crops, including their roots, are destroyed before new nematodes are formed. Short cultivations of lettuce, paksoi, Chinese cabbage and radish, where the roots were removed, suppressed nematode infestation by 50% in research by Cuadra *et al.* (2000). Short summer cultivations of lettuce (cultivation time: 4-5 weeks) also served as trap crops in Dutch greenhouses. This results in many nematodes being caught and year-round lettuce growers hardly experience nematode problems in winter. Growers only growing lettuce in autumn and winter after a fruit vegetable crop, on the other hand, face many nematode problems in winter (cultivation time: approx. 3 months).

Radish species (ölrettich) are currently being used in Germany. Ölrettich (*Raphanus sativus*) is a cruciferous species (Brassicacea) and is interesting in view of a combination of factors, viz. use as trap crop for nematodes, as

biofumigant, and as green manure crop (Melakeberhan *et al.* 2008). As is the case for *Tagetes,* the effectiveness of ölrettich varies per nematode target species. The life cycle of the root knot nematodes must be followed closely for timely incorporation of the ölrettich, viz. before migration of the free-living J2 from the root.

Antagonistic crops have an active controlling effect on plant-parasitic nematodes. There are plants that are killing the nematodes in the roots and there are plants that leak so-called nematicidal substances via the roots. Marigold (*Tagetes* spp.) is an example of both. The active group is a ring-shaped sulphur compound, viz. a thiophenene (e.g. α-terthienyl). As regards effect it is related to metam sodium and prevents the development of juvenile nematodes from the eggs. The substance is activated via light (UV-A) or enzymes (peroxidases) while releasing a reactive oxygen molecule. The substance is by nature used by the plant as defence against chewing. Especially roots contain a high concentration, which increases with the plant approaching the reproductive stage (see §4.3). Generally, the production of so-called secondary metabolites (substances not directly involved in growth such as defence substances) demands energy from the plant; this means that optimum growing conditions result in a higher concentration of secondary metabolites during flowering.

Tagetes spp. have meanwhile for some decades effectively been used in uncovered cultivations against root lesion nematodes (*Pratylenchus* spp.) in, e.g., strawberries. This species has already much longer been in use in India. Recent research confirms that marigold is also effective in greenhouse cultivations against the Southern root knot nematode (*M. incognita*). It is, however, only effective if it precedes a crop. The use of *Tagetes* as undergrowth under vegetables is insufficiently effective in greenhouses.

The cultivars show a wide variation in host plant status for nematodes and effectiveness. It is very important to keep this in mind when using marigold; the cultivars differ enormously in effectiveness, depending on active substance and soil temperature: some cultivars have a wider diversity of thiophenenes; some are most effective at 15°C whereas others are working best at 30°C.

Tagetes is known to have a good effect against the root lesion nematode *Pratylenchus* sp. The root lesion nematode penetrates the root and is then killed. But it is also effective against root knot nematodes. *Tagetes* continually leaks substances from the roots. These substances, in particular thiopenes such as α-terthienyl, are exposed to light and made reactive by soil tillage. The effect of this is clearly shown after rotodigging of the soil: the fields under black fallow were reinfested with root knot nematodes whereas the fields with *Tagetes* showed no reinfestation. The above-ground plant parts do incidentally contain no nematicidal substances and can simply be removed or incorporated. The mode of action of α-terthienyl is similar to that of metam sodium and prevents the development of new nematodes from the eggs.

Most root knot nematodes (*Meloidogyne*) are also killed by certain marigold cultivars but only at soil temperatures between 15 and 30 °C (Ploeg & Maris, 1999a). Especially the roots appear to contain nematicidal substances (see §4.3). Effective cultivars are *T. patula* 'Single gold' and 'Tangerine', and *T. erecta* 'Flor the Muerto'. Cyst nematodes and ectoparasitic root nematodes are not affected by *Tagetes*. *Tagetes* is even a good host plant for some trichodorid nematodes. The plant also acts as host plant for some root knot nematode species or even biotypes (Molendijk, 2000). Root extract of *Tagetes* 'ground control' appears to be an excellent growth medium for *Verticillium dahliae* (Van der Wurff & Paternotte, unpubl. results) and this may mean that 'ground control' is a good host plant for this fungus.

Cultivation of *Tagetes* in the greenhouse, however, is difficult because initial plant growth is slow which gives weed a chance and growth is hindered by rapidly growing production crops such as cucumber or tomato. A crop rotation system such as the Baijens system as described in §4.8, however, is suitable for use of *Tagetes*.

4.4 Natural enemies

Spots are occurring on organic holdings where the nematode population did not increase to high densities or even decreased. This is probably a situation of natural antagonism, although it is not yet known which organisms are involved and how these can be stimulated (see also §4.7). Little effect is expected from the extra application of living organisms that are already present in the soil. Biological control agents that are not originally occurring on the holding may possibly be of some use. This requires that they are capable or surviving, or preferably expanding, in the soil resulting in their latent presence. More than eighty fungi and numerous actinomycetes that may play a role as natural enemy of *Meloidogyne* are known. Examples of fungi are *Arthrobotrys* spp., *Monacrosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Paecilomyces lilacinus*, and *Pochonia chlamydosporia* (e.g. Man-Hong *et al.* 2006).

Several biological control agents have been tested in recent years. Some promising antagonists, such as the bacteria *Bacillus firmus* and *Pasteuria penetrans* and the fungi *Arthrobotrys superba* and *Paecilomyces lilacinus* (Table 15) are discussed below. *Pasteuria penetrans* is one of the most promising control agents which is at the same time very persistent (Van der Wurff 2007; Amsing *et al.* 2006). Each bacterium strain, however, is not equally effective against each root knot nematode species (e.g. Davies & Williamson 2006). A product with *P. penetrans* from Japan had good affinity with *Meloidogyne javanica* and much less so with *M. incognita* isolates from organic holdings. These products require legal authorisation if a controlling effect is claimed.

Table 15. Indeling Classification of the best known biological nematode antagonists according to mode of action.

Plant strengtheners and induced resistance	Nematode-trapping fungi and soil fauna	Parasites of females and eggs
Bacillus firmus *Bacillus subtilis* *Pseudomonas fluorescens*	*Arthrobotrys oligospora* *Arthrobotrys superba* *Dactylaria spp.* *Dactylella spp.* *Predatory nematodes and soil predatory mites*	*Pasteuria penetrans* *Pochonia (Verticillium) spp.* *Paecilomyces lilacinus* *Catenaria spp.*

P. penetrans from Japan is host-specific and with a mortality percentage 95 per cent very effective against, e.g., the root knot nematode *Meloidogyne javanica*. This does require large amounts of bacteria (1 million spores per ml soil). Around 2005 the Japanese have succeeded in setting up a mass culture; until then large-scale culturing of this bacterium was found to be difficult. If this bacterium could establish itself permanently in the soil, it could be a cost-effective control method. The results against the *incognita* nematode, unfortunately, are still inconsistent because the one bacterium population performs better than the other. This is because this bacterium is very choosy and the preference for the type of nematode differs per strain. Another fact is that application of this bacterium is not yet authorised as pesticide in the Netherlands.

The preference of *P. penetrans* (strain Japan) for this type of nematode has been demonstrated in an *in vitr*o test (Figure 11). The root knot nematode (*Meloidogyne incognita*) from a greenhouse of a biogrower (grower B) of greenhouse vegetables went down well whereas a number of other populations (marked PRI-a, PRI-b or PRI-c) left much to be desired. A different test showed that this strain has a special preference for a population of *M. javanica* (Figure 12).

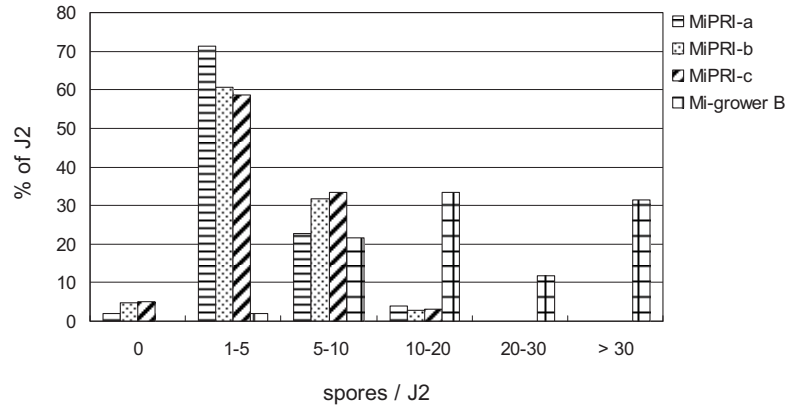


Figure 11. Percentage attachment of Pasteuria penetrans spores to different strains of M. incognita J2. Three strains of PRI have been used (Zoon, pers. comm.) and one population originates from soil of grower B.

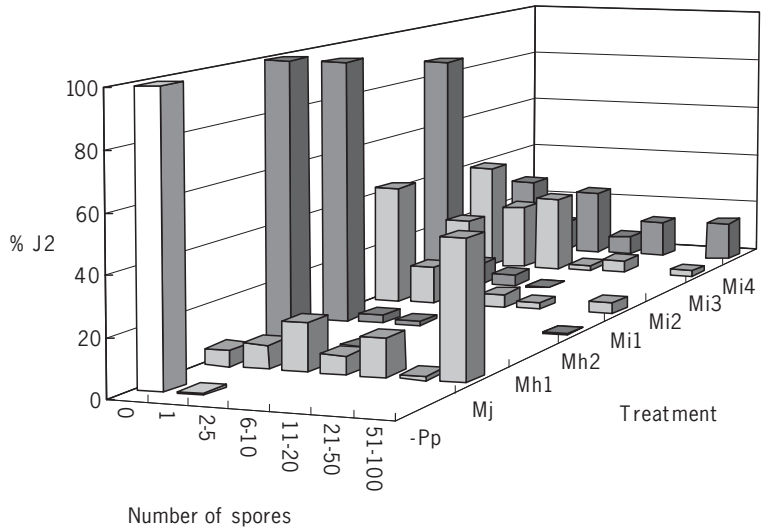


Figure 12. Distribution of the percentages J2 with a certain number of spores of Pasteuria penetrans (strain Japan) correlated to the type of root knot nematode. Mh = Meloidogyne hapla, Mi = M. incognita and Mj = M. javanica. -Pp = (negative control) soil without P. penetrans.

P. penetrans is a bacterium that can only reproduce on nematodes. The resting spores occur freely in the soil. Condition for an effective control result is that sufficient spores of *P. penetrans* are attaching to the skin of second-stage juveniles (J2) of the root knot nematode. When more than twenty spores have attached to the nematode, it becomes very difficult for the nematode to survive. When a nematode with spores would come to infestation and develop into a female, the spores will germinate, infest the nematode where they reproduce. The spores can survive in the soil for years.

In a container experiment with chrysanthemums and the root knot nematode *Meloidogyne javanica* the dose of 1 million spores per ml soil had a controlling effect of 91% after one cultivation round. Lower dose levels showed no effect whereas the percentage J2 with attached spores after the first cultivation round had increased at the lower dose levels. This thus meant an increase in the bacteria population in the soil. Because the (affordable) advisory dose

of appr. 2500 spores per ml soil is too low for directly giving an effective control, this requires the gradual build-up of the bacteria population in the soil.

In 2007 sampling has been repeated at the conventional chrysanthemum holding and at a grower of organic vegetables (Figure 11. grower B) where the field experiments were set up in 2005. A specific molecular DNA qPCR test only detected *P. penetrans* again in the chrysanthemum soil (P. Bonants, pers. comm.; Van der Wurff 2007). This means that the bacterium can survive in the soil for a longer period of time, which brings a cost-effective method within reach. The amount of *P. penetrans* at the time applied in both experiments differed. A larger amount of *P. penetrans* was applied to the soil of the conventional chrysanthemum grower; this may possibly explain why the bacterium was found in the chrysanthemum soil and not on the organic holding.

4.5 Composts

There may be several reasons why application and incorporation of organic fertilisers into the soil has a positive effect on the disease suppressiveness of the soil and thus on crop production.

There may be the following reasons:
1) improvement of soil structure,
2) improvement of the nutrient status,
3) release of substances that are toxic to nematodes,
4) stimulation of the growth of antagonists against nematodes.

Crop residues of the castor oil plant (*Ricinus communis*), e.g., are known to contain nematode-inhibiting substances and also have a repellent effect on nematodes (Zoon, pers. comm. 2005). And other organic fertilisers with similar properties are under development.

Only a marginal nematode control effect is expected of compost and animal manure products that are applied for improvement of soil structure and nutrient status (Kimpinski *et al.*, 2003). Nevertheless, a growth-stimulating effect of the use of compost may be expected as result of an improvement of the physical and chemical soil properties. Research in the past with several types of compost and manure did not show improvement of disease suppressiveness towards root knot nematodes (Amsing & Postma, 2004; Janmaat *et al.*, 2004). Researchers of PPO-BBF in Lisse, however, found a strong suppression of root knot nematodes with an increase in organic matter (Van Os, 2008). Starting point here is that a higher organic matter content stimulates soil life, as regards quantity as well as diversity. This in particular applies for poor soils. Despite the fact that the biomass of bacteria is important, in particular the identity of the species is determinative of the resilience towards fungi (De Boer *et al.* 2003). For nematodes this is unknown. The sandy dune soils that are used for bulb cultivation are characterised by a low (less than 1 %) organic matter content. The project included three organic matter levels: 0.7%, 1.4% and 4% with the integrated cultivation systems on the field with the lowest contents and the organic system on the fields with the higher concentrations. The fields with 4% organic matter show the highest suppressiveness towards root knot nematodes (*M. hapla*; Van Os 2008).

The same fields were included in the soil suppressiveness study of Wageningen UR Greenhouse Horticulture in 2009 and this experiment confirmed that the fields with 4% organic matter (O.M.) had the highest suppressiveness towards root knot nematodes (*M. incognita*) (Figure 13).

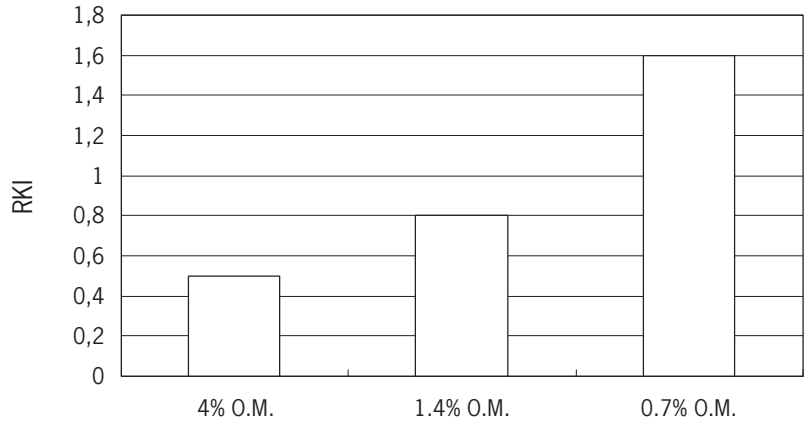


Figure 13. Average RKI (vertical axis) in the cucumber biotest with 4% O.M.; 1.4% O.M. and 0.7% O.M. (O.M. = organic matter). The differences are significant (GLM, P<0.05).

Soil in covered cultivations can show enormous variations in susceptibility to crop pathogenic pests and diseases. On some holdings 100 nematodes/100 cc soil already cause enormous damage whereas such levels cause no damage on other holdings. This also holds for other soil-bound pests and diseases such as *Fusarium, Pythium, Rhizoctonia* and *Verticillium*. A robust measurement system is required to measure differences in soil suppressiveness between soils. Such a system is a necessicity for identifying the cause. This is why reliable soil suppressiveness tests are currently being developed in Bleiswijk. Several measurements such as bacterial and fungal biomass and several enzymatic analyses may serve as indicator for suppressive compost.

4.6 Disease suppressiveness

The dependence on nutrients and phenomena such as '*soil exhaustion*' and pests and diseases easily lead to the soil being seen as a necessary evil. In other words: a complex system of which little is known and which is difficult to control. In particular in greenhouse horticulture, sectors are looking at 'soil-less' alternatives. But traditional uncovered cultivations such as summer flowers, leeks and strawberries are also looking at these systems in view of the increasing problems with the emission of nutrients and chemicals to soil and surface water.

The greenhouse sectors cucumber, tomato, and rose are good examples of a successful change towards 'soil-less' systems. But this does not always offer a solution; the cultivation of chrysanthemums is an example. The first holdings have tried to change over to substrate cultivation but are still facing *Pythium* root rot.

All the more reason to look at '*the way in which nature suppresses pests and diseases*'. This learns how we can make use of the capacity of the soil to suppress pests and diseases and to understand why a pathogen is doing well on one holding and not on the other. And we also learn how we can fight pests and diseases for subsequent application in new cultivation systems.

Various journals present articles about a healthy soil and 'good' soil organisms, whether or not in the context of a commercial product or company. It is important to seriously investigate the scientific evidence on which such articles are based. The discussion about a healthy soil is complex in view of the many aspects. A healthy soil is characterised by the availability of sufficient nutrients for optimum crop growth and by pests and diseases getting little chance. This last property is known as the pest and disease suppressive capacity or suppressiveness.

4.6.1 Definition and origin

Disease suppressiveness means that when a crop is grown on soil or substrate, little damage occurs in the presence of the pathogen (Baker & Cook 1974). Research into pest and disease suppression is not new. We do see, however, an increasing interest of growers who realise that ever fewer (chemical) pesticides are available and that pests and diseases continually come up with a new strategy to escape from suppression.

Pest and disease suppression can be caused by a wide range of mechanisms (Table 16) such as antagonism, where natural enemies can in direct interaction cause a decline in the pest or disease. Examples are the bacterium *Pasteuria penetrans* and the nematode-trapping fungus *Arthrobotrys* spp. and *Dactylella* (see §3.5).
Competition for food may also play an important role, such as competition for biologically available iron between plant-parasitic *Pythium* foot rot fungi and bacteria. Bacteria such as *Pseudomonads* grow much more rapidly and can thus pilfer the important iron elements right under the nose of the fungi. The fact that these fungi have special structures (so-called *siderophores*) for the uptake of, e.g., iron means that this is an important molecule that is not present in excess in the soil as readily available fraction. It is also known that *Trichoderma* fungi grow more rapidly than most plant-parasitic fungi and may thus play an important role in the competition for food in the soil. *Trichoderma* species may differ enormously where some, e.g., do or do not produce antibiotics. In the past, dune sand was sometimes mixed through the compost heap, hoping to enrich the compost with *Trichoderma* species. Another well-known example is the competition for carbon between plant-parasitic *Fusarium* species and non-parasitic *Fusarium* species.

Physical protection of the plant roots is a third way to suppress pests and diseases, where *Trichoderma* species may also play a role (Box 4). Other known species that protect the plant root against parasites are endophytes such as *Glomus* spp. (Mycorrhiza). Unfortunately, these last species are very sensitive to nitrate and phosphate concentrations in the soil and do not play a role in the protection of plant roots in enriched soils. This implies that they are not very important in most agrosystems.

Soil organisms, such as endophytes or bacteria, or certain substances may also increase the suppressiveness of the plant by stimulating resistance-mechanisms of the plant. The literature mentions so-called '*stealth*' as an example. This means that plant roots are invisible to soil pests and diseases. Plant-parasitic fungi grow towards the roots under the influence of a flow of root exudates. Absorbing material in the soil such as the undissolved organic matter fractions and rhizosphere organisms can interrupt the flow of root exudates, thus causing '*invisibility*' of the roots.

In practice the mechanisms of which a number has been mentioned above will act jointly as illustrated by the example of *Trichoderma* spp. These species may switch on possible resistance mechanisms of the plant and physically protect the root against intruders or counteract plant parasites by producing antibiotics.

Table 16. Examples of the various pest- and disease-suppressive mechanisms of the soil.

- Antagonism
- Competition for nutrient elements
- Physical protection of the roots
- Production of antibodies
- Inducing resistance mechanisms
- Making roots '*invisible*'

BOX 4. Trichoderma: an example

The fact that disease suppressiveness of the soil is a complex phenomenon is illustrated by the sometimes apparently unpredictable success of various below-ground natural enemies. The fungus *Trichoderma* sp. is an example. This fungus may be effective against a large number of pathogenic fungi (Mycota) such as *Rhizoctonia, Fusarium, Alternaria, Colletotrichum*, and other fungoids (Oomycetes) such as *Pythium* and *Phytophthora*. It is not always clear why it does in some cases work whereas it does not in other cases. Foot rot (*Fusarium avenaceum*) in Lisianthus (*Eustoma* sp.) is an example. Sometimes it seems to work well in Lisianthus; one time better than the other. Disease suppressiveness is caused by the following mechanisms: a.) suppression by enzymes such as cell wall solvents and antibiotics (antibacterial substances); the fungus is continually excreting these substances and reacts actively to transformation products, b.) suppression by strong competition for food, c.) physical protection of roots, and c.) increasing crop resistance.

These activities depend strongly on soil life and the physical status of the soil, such as bacteria and organic matter. Bacteria may have a direct suppressing effect on *Trichoderma* or an indirect effect by competition with other bacteria for food. Bacteria, such as Pseudomonads, are known for the production of fungicides (also called soil fungistasis). These substances are only produced in the presence of sufficient Pseudosomads (stimulation via DAPG also between species). This means that determination of the presence of these groups in the soil as such is not enough (identity) because activity is the issue, i.e. the production of suppressive substances aimed against the pathogen (function).

The effect of *Trichoderma harzianum* against *Fusarium* foot rot in bio-Lisianthus is less in organically than in conventionally (i.e. with pesticides) raised *Lisianthus*. The producer of *Trichoderma* found a wider diversity of bacteria on the bio-Lisianthus; this may indicate an (in)direct effect by competition for food or by toxic bacterial substances. Despite the fact that *Trichoderma*'s are known for their rapid metabolism in comparison with other fungi, they can generally not handle the competition for food with bacteria. This last fact is also related to the amount of organic matter. Organic matter is the engine, the energy supply, of the soil on which many soil processes depend. Especially bacteria react to the addition of organic matter with a rapid increase in numbers. Fungi have fewer chances under intensive soil tillage and bacteria are rapidly taking over. Summarising, the success of *Trichoderma* depends on several factors, such as physical status of the soil, the degree of soil tillage, organic matter, pesticides, and bacterial community.

4.6.2 Research into suppressiveness

Research into suppressiveness can be divided into diverging and converging. The first type of research investigates natural enemies of pests and diseases and tries to understand how we can create a suppressive soil. Converging research studies the variation in organisms and physical status in natural soils and links this to pest and disease intensity. The good thing of this last aspect is that growers are directly involved in this research, i.e., their soil is turned upside down and analysed (Figure 14). Both research directions are important for understanding the complex relationships between plant parasites and their environment and for developing a sustainable suppression strategy. Pest and disease suppression by the soil is complicated because soil life as well as physical soil parameters are playing a role.

Converging research is carried out in organic vegetable cultivation. Earlier results on the soil of twenty bio-growers shows that soil suppressiveness against root knot nematodes (Figure 14) or *Fusarium oxysporum* can vary enormously. This turns them into an attractive experimental gradient. Soil is collected and analysed for suppressiveness towards a number of pathogens, such as root knot nematodes (*Meloidogyne* sp.), *Pythium* spp. and *Verticillium* (Schreuders & Van der Wurff 2009). The soil samples are at the same time analysed for parameters that may be relevant such as fungi, bacteria, physical properties, etc. Several institutes are participating, all with their own expertise. Possible causes are then investigated by searching for a relationship between suppressiveness and fungi, bacteria, physical properties, etc. Factors that are relevant for suppressiveness are then translated into (optimisation of) cultivation measures.

A hypothetical example of this is that an increase in labile organic matter, combined with a high acidity and magnesium concentration, may result in a strongly reduced chance of loss caused by foot rot (*Fusarium avenaceum*).

There is a lot of new attention in international research for disease-suppressing soils (Van der Putten 2006; Kerry 2002). These are soils where pests and diseases have no chance and can suppress an introduction of harmful nematodes or fungi. These properties have indeed been found in a number of bio-greenhouses (see Figure 14). This phenomenon can be explained by the fact that in these soil a very large number of different actors, living and not living, together prevent the development of a pathogen. Much is still unknown about such mechanisms and how such properties can be stimulated in the soil. Work on this will be carried out in the years ahead (in particular the application).

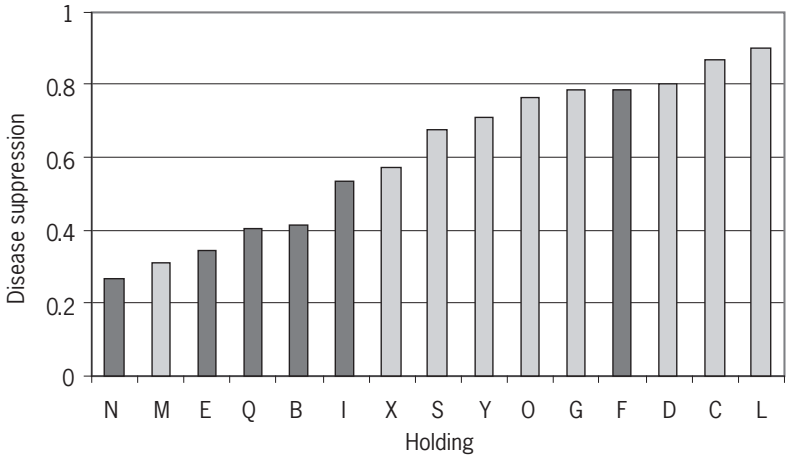


Figure 14. Difference in suppressiveness of organic holdings against the root knot nematode M. incognita (From: Berkelmans & Termorshuizen 2005). Dark grey shading means that the soil has been soil steamed less than 2 years before sampling.

Pest and disease suppressiveness does exist. Despite the fact that this is a complex phenomenon, our understanding of its mode of action is continually improving. We search for a mechanism that gives an explanation. A mechanism that can be converted into cultivation measures for different soils. Research within the different sectors is important to investigate whether there is a different disease suppressing mechanism for different soils and crops, such as vegetables or cut flowers. The effect of pesticides is also important because these products are still an important weapon in the fight against pests and diseases. Increasing soil suppressiveness decreases the chance of pests and diseases. It does not guarantee cropping without problems. Organic systems are characterised by fluctuations in natural enemies and prey and an absolute removal of a pathogen from a system is an illusion. But we can strive for a reduced use of environmentally unfriendly pesticides by utilising the competence of the soil.

4.7 Alternative soil disinfestation

Various alternatives are available for chemical soil disinfestation. These serve to bring the initial infestation before planting down below the economic damage threshold. Alternatives are: steaming, solarisation, anaerobic biological soil disinfestation (BSD), inundation and biofumigation. The killing effect of steaming and solarisation is achieved via heating of the soil; BSD and inundation involve the creation of anaerobic conditions in the soil resulting in the formation of nematode-killing substances, such as propionic- and butyric acid, by the decomposition of organic matter.

Biofumigation is making use of the fact that certain types of organic material yield gaseous substances with a nematicidal effect during the degradation process within soil. It is known, e.g., that incorporated glucosinolate-containing crops release isothiocyanates, a substance capable of killing nematodes (Stirling & Stirling 2003; Zoon, 2004, McSorley *et al.* 1997).

Black fallow also is an effective method for suppressing nematode populations. The effect of fallow depends on the type of nematode, level of the initial infestation, soil temperature and moisture, duration of the fallow period and a good weed control. Here good weed control is defined as keeping free from weeds that may serve as alternative food. To make the fallow period more effective, it may be useful to remove infested roots from the soil as much as possible. The extent to which the nematode population decreases under greenhouse conditions is unknown but a period of six months will certainly be needed for reaching a reasonable effect.

Meloidogyne incognita and other heat-demanding species are in particular decreasing at temperatures above 20 °C and below 10 °C (§1.1.3). The moderate species (such as *M. hapla*) remain present for a long time at low temperatures. Fallow may be more effective in combination with covering with plastic (solarisation) because the nematodes loose more energy at higher temperatures. In the open field a decrease of approx. 85% is assumed after a year. Weeds must not only be removed during the fallow period but this is also essential during the cultivation of non-host plants, resistant host plants and antagonistic trap crops to avoid weeds nullifying the effect of these crops.

But the speed of the succession of cropping rounds is one of the problems. There is not much time for lengthy soil disinfestation. The greenhouse is only empty for a few weeks in December but soil temperatures are very low during this period.

4.7.1 Biofumigation

Biofumigation is the method that seems to offer perspectives for the control of the root knot nematode. Biofumigation (McSorley *et al.* 1997; Sarwar *et al.* 1998) is a form of soil disinfestation utilising substances that are released by chopping fresh brassicacea such as mustard. Use is made of the natural defence reaction of cabbage plants after chewing. Damaging of the cells results in the release of very toxic substances. The effectiveness of biofumigation, however, is not always equally good because this depends on factors such as cultivar, cropping conditions such as soil type and climate, time and method of incorporation, and possibly also adaptation of the target species. It is known that the action of these gaseous isothiocyanates is similar to that of Vapam (metam sodium; Tsao *et al.* 2002). Prolonged use of Vapam could result in adaptation of the target pathogen, with the result that the target pathogen does not react to biofumigation either (as discussed for *M. hapla* by Melakeberhan *et al.* 2007).

Biofumigation has the advantage that 'cabbage-type' plants can be grown on the holding and that this meets all criteria for organic cultivation. Disadvantages, however, are that soil incorporation is very labour-intensive and that there is a waiting period of about ten days after incorporation; this is because the thiocyanates are also toxic to the crop. Another disadvantage is that the incorporation of crop residues may also bring other nematodes and fungi into the greenhouse which entails the risk of a new source of infestation being introduced. This is why several market parties are currently working on mustard plant extracts in the form of granules and extracts in liquid form.

Most cruciferae (such as Brassicas) contain sulphur-containing glucosinolates; these are after bruising converted -by means of the enzyme myrosinase- into reactive isothiocyanates (*the spicy mustard taste*) that are lethal to nematodes in high concentrations (Zoon *et al.* 2004). Mustard products can also be obtained in dried or liquid form. The effect of these concentrated extracts under glass needs further investigation. Cultivation of sufficient biomass (40-50 t/ha) at low winter temperatures in the greenhouse requires 3-4 months and proceeds much faster in summer. Nematode resistance is required when such crops are grown in the greenhouse over a longer period but cultivation outside the greenhouse is also an option; this requires no resistance. Various other antagonistic crops have been studied in other parts of the world, including some tropical *Crotalaria* species that are resistant to *Meloidogyne* and – in addition – give extra mortality after incorporation (Wang et al 2002). *Crotalaria juncea* can in two (summer) months

produce sufficient fresh biomass for a substantial suppression of the nematode population. In addition, this cruciferous crop can via nitrogen fixation contribute to the mineral balance sheets (Duke, 1981; Roseberg, 1996).

After chopping and incorporation some green manures or crops release toxins that kill nematodes at higher concentrations. These toxins must therefore be preserved by airtight covering of the soil with transparent plastic foil (Figure 15). Must cruciferae contain sulphur-holding glucosinolates that are after bruising transformed into reactive isothiocyanates by means of an enzyme (*myrosinase*; Van Eylen *et al.* 2006). This mechanism is based on a natural defence reaction of the plant. As soon as the cell structure of the plant is disturbed two components come together, producing a toxic and gaseous substance. This principle is used by mowing, chopping and direct soil incorporation of the mustard plants. The speed of chopping and incorporation is important. Another fact is that, as for marigold, the component concentration is highest during flowering. After incorporation the soil is covered with sunlight-transmitting foil. The whole is then left alone for 8-10 days.

In a greenhouse experiment in Bleiswijk mustard was grown in a greenhouse after a heavy infestation of the soil with *Fusarium avenaceum* in Lisianthus. Large parts of the crop had been lost due to *Fusarium avenaceum* before the mustard could be incorporated. This means that the cultivation of mustard in the greenhouse may hold a high risk.

As for *Tagetes*, the nematicidal effect varies strongly per cultivar. At the moment Sarepta mustard (*Brassica juncea*) is most frequently used. An additional advantage of growing mustard and *Tagetes* as inter-crop is that they strongly reduce the amount of nitrate in the soil.

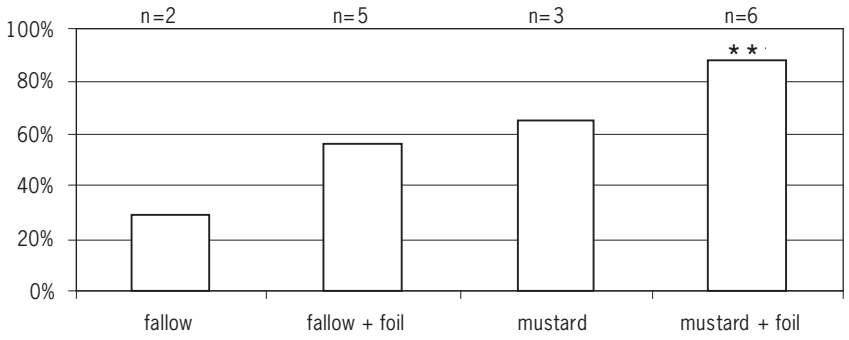


Figure 15. Percentage mortality of Meloidogyne spp. by biofumigation in a field experiment with 50 t fresh weight yellow mustard (Sinapis alba) per ha in the period December - April. The treatment 'mustard + foil' gave a higher mortality than 'black fallow', 'black fallow + foil', and 'yellow mustard without foil'. 'n' indicates the number of replicates per treatment.

Verticillium dahliae is a problem in organic vegetable cultivation, mainly in sweet pepper. This pathogen is difficult to control. Soil steaming is at the moment the only effective means but growers do not like this method in view of the many negative side-effects such as the decrease in natural soil suppressiveness towards *V. dahliae* and other pathogens and pests.

Blok *et al.* (2000) achieved good results in controlling *V. dahliae* by means of biofumigation (breaking of the cell structure brings the enzyme myrosinasis into contact with glucosinolates and converts these into gaseous toxic isothiocyanates) with broccoli and anaerobic soil disinfestation with grass. The results in own research with biofumigation (with broccoli) were less favourable (Figure 16). This can be explained by the difference in type of cultivar or freshness of the broccoli. The same experiment, conducted on a second commercial holding with other grass and broccoli, neither showed an effect in comparison with the controls (*not presented*). This may have been caused by differences in the execution of the experiment but also by differences in soil composition. The effect of soil type on the effectiveness of BSD is further investigated in running research in LNV BO-04. In addition, several new mustard cultivars, with a high glucosinate content, are now available and can be tested.

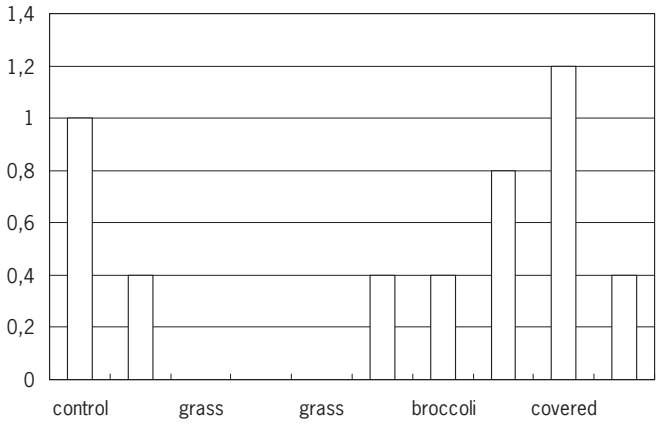


Figure 16. *The graph shows the average V. dahliae disease index over 5 plants (n = 5) in an biotest. The treatments were carried out in cement barrels dug into the soil on a commercial holding. The biotest was then carried out in Bleiswijk with P9 pots and sweet pepper plants for 6 weeks. The disease index was determined per plant as the sum of 5 typical symptoms, i.e., black stem base; colour (dark vs. light leaves); wilting (especially bottom leaves); unbalanced growth of leaves, and dying off.*

4.7.2 Anaerobic soil disinfestation

Several plant parasites have a poor resistance towards anaerobic conditions. The control mechanism behind biological soil disinfestation, however, is not clear but probably is a combination of anaerobic conditions, harmful products released by anaerobic decomposition such as ammonia, and natural enemies that flourish under anaerobic conditions. This is why the term Biological Soil Disinfestation (BSD) is frequently used.

Blok *et al.* (2000) were the first in the Netherlands to bring this knowledge into practice with a field-scale experiment in which biofumigation was tested besides anaerobic soil disinfestation against several soil pathogens, viz. *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *asparagi*, and *Rhizoctonia solani*. The authors documented a strongly suppressive effect of anaerobic conditions. Products such as carbon dioxide, ethylene, hydrogen, methane, ammonia, organic acids, alcohols, and aldehydenes are formed under anaerobic decomposition and some are known to have a fungicidal effect. And various antagonists are known as typically occurring under anaerobic conditions, such as *Bacillus* spp. and *Clostridium* spp. (Blok *et al.* 2000).

The lethal effect of biological soil disinfestation on nematodes is even better than on soil fungi. In various experiments by PPO-AGV the lethal effect on root knot nematodes (*M. fallax*), root lesion nematodes (*Pratylenchus penetrans*), and potato cyst nematodes (*Globodera pallida*) was qualified as good but the effect on (virus-transmitting) trichodorids was variable.

It was also found that in practice anaerobic soil disinfestation seems to be effective against loss of asparagus by *Fusarium*, despite the fact that *Fusarium* remained present in the soil after disinfestation. Strengthening of the general microbial activity in the soil, by incorporation of grass, is sufficient to prevent plant loss caused by pathogens. Blok *et al.* (2000) describe a clear decrease of microsclerotia of *V. dahliae* by anaerobic soil disinfestation.
The effectiveness of anaerobic soil disinfestation against *M. incognita* (Paternotte *et al.* 2009) has been tested on two organic vegetable greenhouse holdings. In October 2008 grass was incorporated down to a depth of 30 cm and covered with special airtight plastic. Nylon bags with infested root material were dug into the greenhouse soil at ten places and soil samples were taken for nematode analysis before the soil was covered. Fresh soil samples were taken after 6-9 weeks and the nylon bags with infested root material were collected for analysis of the survival of root knot nematodes.

Soil temperature during the anaerobic soil disinfestation was 19 - 20 ºC on one holding and about 14 ºC on the other. Anaerobic soil conditions were already reached within a few days after the soil was covered with plastic.

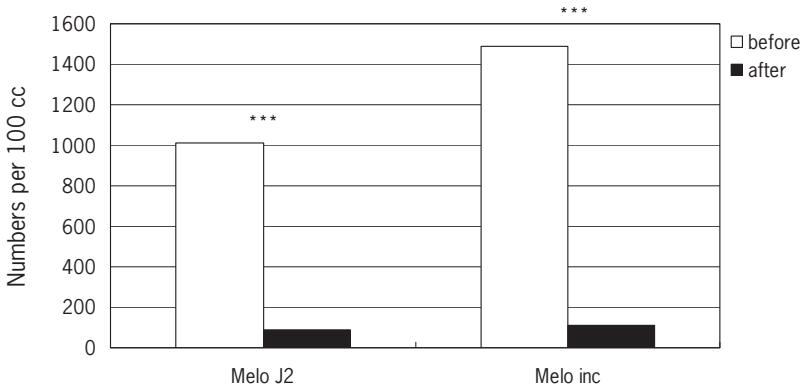


Figure 17. Numbers of free-living (J2) Meloidogyne in the soil before and after anaerobic soil disinfestation. 'Melo inc' represents the number of nematodes in egg masses (incubation fraction; soil incubated for 28 days to make sure that the largest possible number of nematodes had hatched from the eggs to be included in the counts).

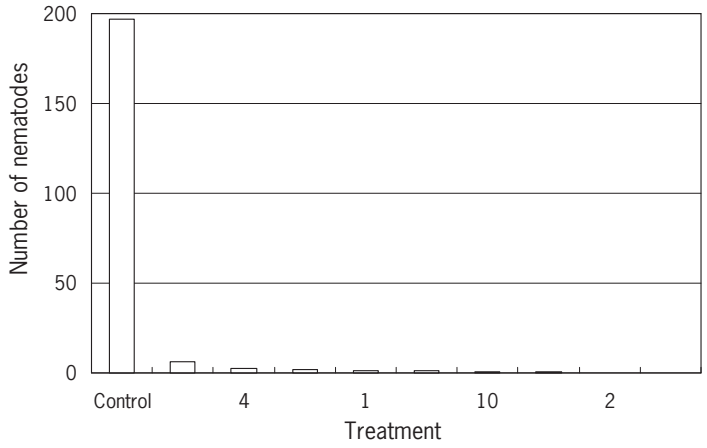


Figure 18. Numbers of Meloidogyne in the dug-in nylon bags (1-10) after anaerobic soil disinfestation in comparison with the untreated control.

Anaerobic treatment was very effective on one holding where the number of nematodes in the soil samples (Figure 17) as well as in the root material (Figure 18) decreased by 90%. On the other holding, however, the number of root knot nematodes in the soil did decrease strongly but this was not the case in root material (*results not presented here*).

Figure 19 shows that fungus- and bacteria-eating nematodes (*so-called* saprophytes) also decrease in numbers from about 5000 to 3000 individuals per 100 cc soil. These nematodes are not harmful to the crop and play an important role in the transformation of organic matter into nutrients (decomposition). The numbers of saprophytes before and after BSD in the incubation fraction show no significant differences. This, however, is less relevant and also logical because the soil is incubated for 28 days to let the *Meloidogyne* egg masses hatch, which means that the numbers can increase again (see Figure 17 'Melo inc.').

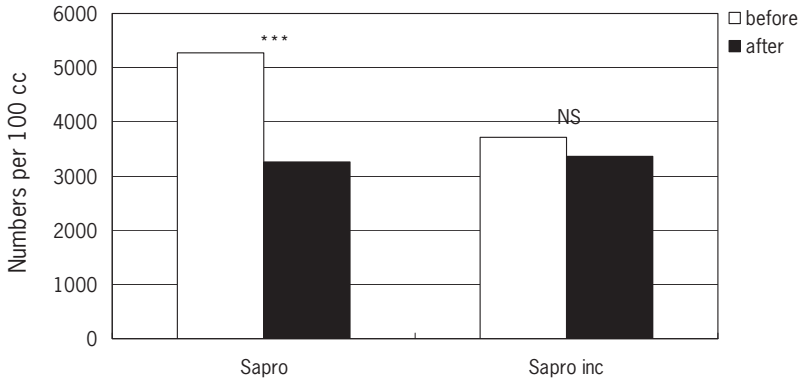


Figure 19. Difference in numbers of saprophytic nematodes (bacteria- and fungus-eaters) in the soil before and after anaerobic soil disinfestation as indicator of beneficial soil life. 'Sapro inc' represents the number of nematodes in egg masses (incubation fraction; soil is incubated for 28 days and nematodes are flushed again and counted).

The cause of the difference in effectiveness of the treatments between the holdings is not clear. The temperature of the soils seems to have played a role. But the difference in effectiveness of the anaerobic soil disinfestation can also be explained by, e.g., a difference in present natural enemies or ammonia gas formed during anaerobic processes.

Alternative anaerobic soil disinfestation is currently being developed where a fermented product is scattered over the soil and covered for 3 weeks (Runia *et al.* 2009).

4.8 Crop rotation with soil disinfestation against nematodes

How can space and time be made for an alternative disinfestation in summer, and without production loss? Organic growers Gebr. Verbeek in Velden (Limburg) and Wageningen UR Greenhouse Horticulture found a solution in an unconventional cultivation system (Van der Wurff & Berkelmans 2009). Instead of planting all beds with cucumber, one of each two adjacent beds is skipped. A double number of plants is planted to prevent production loss and the plants are guided over the empty bed as a pergola by using long wires. This compensates the loss of production for the restricted area that has been planted. The system does, incidentally, only work for cucumber. Sweet pepper is difficult to bend and tomato is growing too slow. Organic grower Mar Baijens (Velden, the Netherlands) has already been using this system successfully for a longer period and was also interested in an experiment in which the effect on nematodes and cucumber production would be monitored accurately.

The results of the experiment on field scale were very encouraging. The result improved considerably after the disappointing production of the pergola-wire system in the first cultivation round (Figure 20). Nematodes increased considerably throughout the year in the conventional cultivation system and this resulted in a high production loss. The pergola-wire system, however, was much less affected by this and yielded the same production as the conventional system in the second as well as the third cultivation rounds. This means that the effectiveness of the *Tagetes* soil disinfestations is a decisive factor in order to increase production levels towards those of a cultivation without nematode damage.

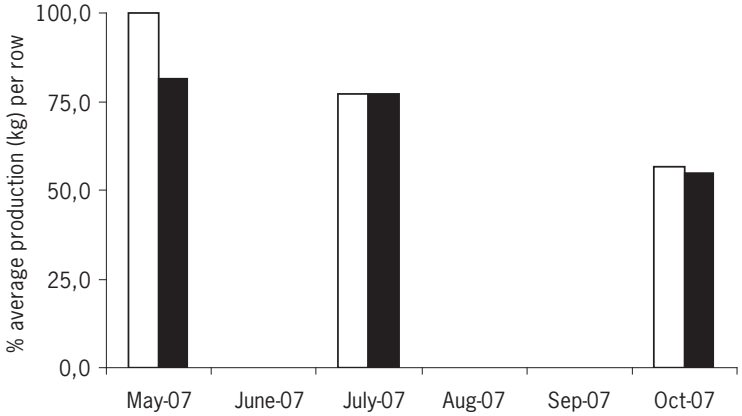


Figure 20. *Production of the Baijens pergola-wire system (■) in comparison with the conventional cultivation system with larger nematode damage (□). Production is given as percentage in comparison with the production of the conventional system in the first cultivation round (May-07 = 100%).*

Photo 3. *Baijens system with cucumber and Tagetes as inter-crop.*

A prolonged effect on root knot nematodes in particular exists after termination of the cultivation of *Tagetes* (Figure 21A). The fact that this is not an aggressive control agent becomes clear after a cucumber crop. There is little effect on nematodes (Figure 21B). This was also caused by the poor growth of *Tagetes* in May by water deficiency, reduced seed germination by chewing slugs, and a short cultivation round.

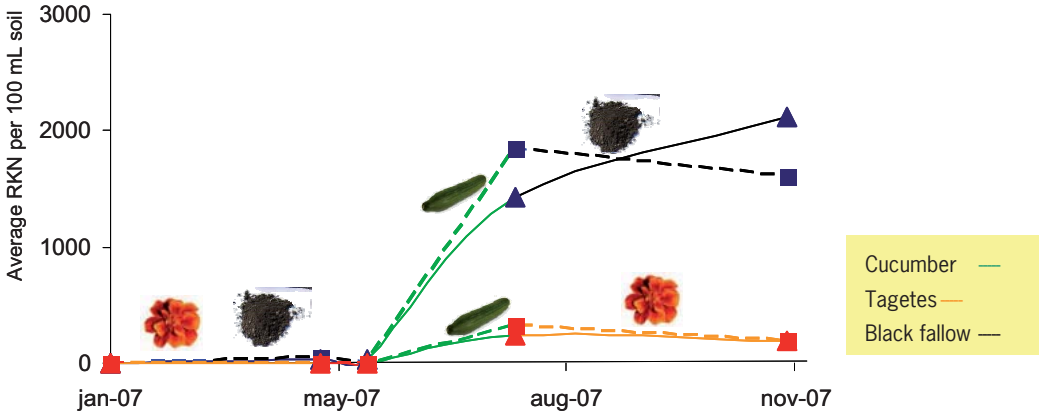
Tagetes results in less damage to the cucumber roots in all cultivation rounds. The root knot index is a full point lower. This is important because root damage causes poorer water uptake and transport, and thus a lower yield. Moreover, significantly better quality (more class 1) cucumbers were harvested in the third cultivation round.

Generally, *Tagetes* has a positive effect on soil life and on disease suppressiveness of the soil by stimulating good (root) bacteria. Unfortunately, the introduction of new plants in the greenhouse often also introduces new pests and diseases.

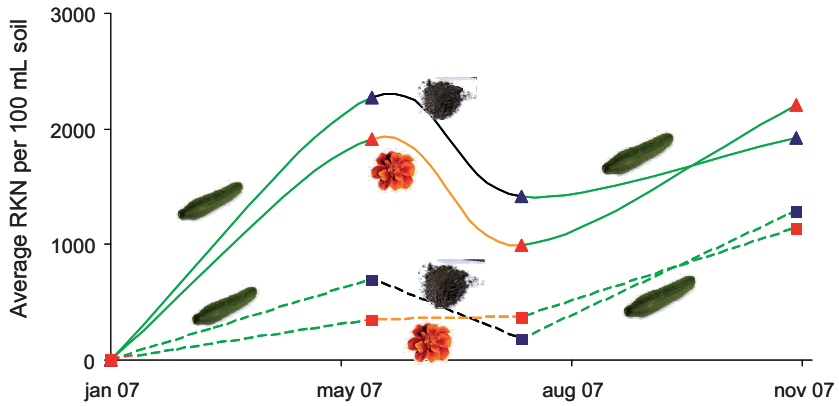
Table 17. Tagetes species on which nematodes can reproduce and that cannot be used in the pergola-wire system.

Species		Cultivar	Meloidogyne species
Tagetes	*erecta*	Carnation	*hapla*
Tagetes	*erecta*	Diamond Jubilee	*arenaria*
Tagetes	*erecta x patula*	Polynema	*arenaria*
Tagetes	*patula*	Goldie	*incognita, arenaria, hapla*
Tagetes	*patula*	Petite Gold	*incognita, arenaria, hapla*
Tagetes	*patula*	Petite Harmony	*incognita, arenaria, hapla*
Tagetes	*patula*	Petite Harmony	*arenaria*
Tagetes	*signata pumila*	Golden Gem	*incognita, arenaria, javanica*
Tagetes	*signata pumila*	Tangerine Gem	*incognita, arenaria, hapla, javanica*

The type of root knot nematode in the soil must also be taken into account. The cultivar *Tagetes patula* 'Petite Gold' is a good host plant for the nematode *Meloidogyne hapla* (Table 17). Information about the types of root knots that are present in the soil is therefore important.



A.)



B.)

Figure 21. *Course of the average number of free-living root knot nematodes in fallow (▲) and Tagetes plots (▲), and number of nematodes in eggs in fallow soil (■) and Tagetes plots (■) in the pergola-wire system. A.) From January, after soil steaming, until May the bed was kept fallow, or Tagetes (Tagetes patula 'Ground Control') was planted. From May cucumber was grown on these beds and from the end of July again followed byTagetes or fallow. B.) Cucumber was grown on the bed from January, after steaming, until May. From May these beds were kept fallow or planted with Tagetes (Tagetes patula 'Ground control') before again planting cucumbers from the end of July.*

The Baijens pergola cultivation system is a first step towards a root knot nematode suppressive cultivation system. The results are promising. Only in the first cultivation round yields of the pergola-wire system are lower than in a conventional cultivation system. This is a pity because these cucumbers are most profitable. Production of the conventional and alternative cultivation system is the same in cultivation rounds two and three. This means that in summer the system offers room for prolonged soil disinfestation without extra production loss whereas this is impossible in a normal system (Box 5). A better cultivation of *Tagetes* in round two may result in a higher cucumber yield in cultivation round three. Poor growth of *Tagetes* in the summer months resulted in a disappointing nematode suppression in the third cucumber cultivation round.

BOX 5	
Advantages: • Room for alternative soil disinfestation in summer without loss • More class 1 cucumbers • Less nematode damage to roots • Lower numbers of nematodes in the soil • Increased suppressiveness of the soil	Disadvantages: • A loss of 20% in the 1st round • Harvesting and leaf treatments difficult • Damage to fruits above the path by electric trolley • Possible nutrient deficiency by double planting

Tagetes appears effective against root knot nematodes, provided it is grown prior to a production crop. Other disease-suppressing plants that may be used are also investigated in running research.

Less positive findings of the cultivation system until now are that workers are finding it hard to harvest above their heads. This also makes leaf treatment with biological products difficult. And the larger number of plants per square metre may sooner result in nutrient deficiency.

5 Discussion and conclusion

Finding a sustainable solution for crop damage caused by root knot nematodes (*Meloidogyne* spp.) has been the main aim of this research. The study focused on the development of alternative control systems for root knot nematodes with the least possible damage and making steaming unnecessary. This project has actually led to the testing of a wide range of control options in close cooperation with growers. Most options, such as biofumigation and Biological Soil Disinfestation (BSD) had never been tested in greenhouse soils. The close cooperation with the organic growers results in new options rapidly finding their way into practice and in growers gaining practical experience. This project resulted in a wide range of options growers can choose from to create location-specific control solutions for the root knot nematode problem.

5.1 New cultivation systems

The ideal solution would be a cultivation system in which root knot nematodes have no chance, such as in substrate cultivation. Substrate cultivation, however, is no option in organic cultivation. The Baijens cucumber cultivation system is getting close but it is a compromise: it creates extra room in the greenhouse for prolonged alternative soil disinfestation (at least one cultivation round) but this is only interesting in case of high root knot nematode damage. This system makes it possible to make room for a soil disinfestation with *Tagetes*, black fallow or BSD without production loss in comparison with a system with nematode damage. But without nematode damage the system means a loss. Intercropping with plants such as *Tagetes* is not without risk because pests and diseases such as the soil fungus *Verticillium dahliae* or harmful nematodes may be introduced. Indirectly, diseases such as *Botrytis* may strike as result of the change in the local greenhouse climate resulting from intercropping. Research into alternative crop rotation systems (see also LNV project Bio-rotation Greenhouse) shows that a change in cultivation system is not easy because in a rotation system the number of plants per surface area is higher, which has consequences for fertilisation and watering strategies.

5.2 Soil amendments, plant strengtheners and PNOs

Many products have been tested but by far the largest part showed no or little effect against root knot nematodes or is not authorised. Part of the products were supplied via the growers themselves or by commercial parties and part were prepared by ourselves on the basis of information from the international literature. The results show that an eye for detail is important when using these products. Effectiveness of products may depend on target nematode, especially for biological control agents such as *Pasteuria penetrans*. But soil type, soil life and crop type are important factors as well. The cause of product failure is often obscure. An example has been described for BSD and biofumigation where a large difference in effectiveness of *Verticillium dahliae* against root knot nematodes was found between two holdings. Processes such as biodegradation may also play a role. This means that soil life is (too) rapidly transforming products into a harmless variant. A known example from conventional cultivation is fenamiphos against nematodes.

Fertilisers such as Agri Biosol and borium require an optimum concentration. Our research clearly showed a negative effect of a 20 cc application of Agri Biosol whereas a 10 cc application caused no problems with root knot nematodes. Excess borium stimulates root knot formation whereas a lower dose causes repellency of root knot nematodes. It is therefore important for growers to conduct an ‘on-the-spot’ pre-test to investigate the consequences before starting full treatment.

5.3 Phytochemicals

Cultivar identity and harvest timing of plant parts play an important role in the case of plant extracts. The literature frequently states that production of secondary plant substances is highest in the flowering period. A number of plant

extracts have been tested in the laboratory, part of which showed a controlling effect on root knot nematodes. Extract (PPO-X) is the only product that has also been tested in soil where it showed a controlling effect, but this was inadequate.

5.4 Alternative soil disinfestation

For the time being, the effectiveness of *Biofumigation* as soil disinfestation appears unpredictable due to the dependence on plant identity, harvest moment and speed of incorporation. And Sarepta mustard (*Brassica juncea*) appears to be a good host plant for *Fusarium avenaceum*. This may possibly enhance *F. avenaceum* problems in organic freesia and lisianthus rather than controlling them. BSD is also labour-intensive but is earlier to standardise, which increases the predictability of the killing effect. Other parties are currently working on a faster and more effective method in which grass is replaced by a powder. An effective plastic soil cover remains crucial for these methods.

5.5 Composts

The effect of compost is promising but the use of nematode-suppressing compost is for the time being difficult to introduce in practice. Steering compost towards suppressiveness requires accurate monitoring of the composting process with an eye for temperature, bacterial transformation and diversity, organic matter quality (biologically degradable fraction), and the decomposition process. This means that no high hopes should be held of compost *per se* and that research cooperation with a compost supplier is important to arrive at a predictable disease-suppressing product. Nevertheless, the results of the Topsoil project in Lisse (PPO-BBF) yield promising results.

5.6 Conclusion

The study shows that a *one-option-fits-all* strategy does at the moment not yet exist and that the solution consists of a package of measures to be chosen from, depending on target pathogen, crop, type of holding and (a)biotic soil composition. Thanks to this study there now is a practical overview of available control options with advantages and disadvantages and numerous new approaches have been formed for solving the pest and disease problems in soil-bound cultivation under glass.

The new LNV soil suppressiveness research by WUR Greenhouse Horticulture (Soil Advisory System within BO-04 and BO-06) provides a good basis for gaining more insight into differences between greenhouse soils and why one product or method such as Biofumigation or BSD is effective on one holding but not on the other. The study comprises comparison of soils for various characteristics such as bacterial and fungal biomass, diversity of Pseudomonads, Streptomycetes, Nematodes and several physical and chemical properties. A link can easily be made by looking at the effect of various products and methods on soil suppressiveness. This work is aiming at the development of an integrated soil advisory system, which enables soil suppressiveness management by means of cultivation measures (such as e.g. biofumigation or BSD).

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Annex I.

Root Knot Index

0. No knots

1. Some small knots, difficult to find

2. Small knots, clearly visible

3. Some larger knots

4. More large knots

5. Knots on 25% of the roots

6. Knots on 50% of the roots

7. Knots on 75% of the roots

8. Knots on 90% of the roots

9. Knots on 100% of the roots; Plant dies

10. All roots with knots; hardly roots left; Plant is dead

Annex II.
Overview effectiveness against nematodes

Name (manufacturer)	Authorised*?	Effectiveness in soil	Field/pot experiment	Target nematode (if known)	Remarks	Reference
Agro Biosol	Y	-	Field/Pot	*M. incognita; Meloidogyne* spp.†	Excess levels stimulate root knots.	Fig. 3, 7
Allium sp. plant extract	Y	?	Lab	*M. incognita*		Fig. 9
Bacillus subtilus	N	-	Pot	*Meloidogyne* spp.†		Fig. 5
Biological Soil Disinfestation (BSD)	Y	+++	Field	*Meloidogyne* spp.†	Difference in effectiveness between grasses and other products; provided careful execution/ covering. Effect on *V. dahliae* microsclerotia uncertain.	Fig. 17,18, 19
BioAct®WG (Prophyta)	N	-	Fleld/Pot	*Meloidogyne* spp.†	*Paecilomyces lilacinus* Strain 251 (PL 251). Preference for *M. hapla likely*.	Fig. 3, 7, *not presented*.
Biofumigation	Y	+++	Field	*Meloidogyne* spp.†	Difference in effectiveness between brassica species; provided careful execution/covering.	Fig. 6, 15, 16
Biopol product X (Biopol)	N	-	Pot	*M. incognita*		Fig. 3
BMS (BMS)	Y	-	Field/pot	*M. incognita*		Fig. 3, *not presented*
Borium	Y	+	Pot	*M. incognita*	Root knot increase at excess levels.	Fig. 3
Caliente (PHC)	Y	+	Field/pot	*Meloidogyne* spp.†	Provided irrigated into the soil and covered. Results not consistent.	Fig. 5, 6, 7, *not presented*
Capsanem (Koppert)	Y	-	Pot	*Meloidogyne* spp.†	*Steinernema carpocapsae*	Fig. 5
Lemon grass	Y	?	Pot	*Meloidogyne* spp.†	Chopped and incorporated. Generally available.	Fig. 7
Entonem (Koppert)	Y	-	Pot	*Meloidogyne* spp.†	*Steinernema feltiae*	Fig. 5
Ginger oil	Y	+	Pot	*Meloidogyne* spp.†	Incorporated. Generally available.	Fig. 7

Name (manufacturer)	Authorised*?	Effectiveness in soil	Field/pot experiment	Target nematode (if known)	Remarks	Reference
Glomus sp.	Y	-	Pot	*Meloidogyne* spp.†	Mycorrhiza	Fig. 7
Helenium sp. plant extract	Y	?	Lab	*M. incognita*		Fig. 9
Melogone II (DCM)	N	-	Pot	*Meloidogyne* spp.†		Fig. 3
Microbial combi DK	Y	-	Field			*Not presented*
Nutrineem (NTS)	Y	-	Field	*Meloidogyne* spp.†		Fig. 6
Pasteuria penetrans (Japan)	N	+++	Field/lab	*M. javanica*, some populations *M. incognita*		Fig. 11, 12
Prosper Nema (Biopol)	Y	-	Field	*Meloidogyne* spp.†	Mycorrhizae	Fig. 8
PPO-V (WUR)	N	?	Lab	*M. incognita*	Plant extract	Fig. 9
PPO-W (WUR)	N	?	Lab	*M. incognita*	Plant extract	Fig. 9
PPO-X (WUR)	N	+	Pot/lab	*M. incognita*	Plant extract	Fig. 3, 9, 10
PPO-Y (WUR)	N	?	Lab	*M. incognita*	Plant extract	Fig. 9
PPO-Z (WUR)	N	?	Lab	*M. incognita*	Plant extract	Fig. 9
Product X (Koppert)	N	+	Pot	*M. incognita*	-	Fig. 3
Prosper Nema (Biopol)	Y	-	Field	*Meloidogyne* spp.†	mix of Mycorrhizae spores.	Fig. 8
Ricinus scrap (*Ricinus communis*)	J	-	pot	*Meloidogyne* spp.†	Biofumigation	Fig. 5
Rincasa (PRI)	N	+	Pot	*Meloidogyne* spp.†	Experimental product until 2008; development discontinued.	Fig. 5, 7
Tagetes evergreen plant extract	Y	?	Lab	*M. incognita*		Fig. 9
4 or 8 °C (eight days soil incubation at 4 °C)	Y	-	Pot	*Meloidogyne* spp.†	Both temperatures have no effect on survival.	Fig. 7

* *= legal authorisation as pesticide is required if an effect against root knot nematodes is claimed or if used as such;* † *= Meloidogyne spp. refers to a mix of several species without the species being known. This was especially the case in field experiments but also in some pot experiments on commercial holdings where well-mixed greenhouse soil was used.*

Annex III.
List of Figures

Annex IV.
List of Tables

Annex V.
Overview host plant status flowers

X	Host plant	X/N	Species- or cultivar-dependent host plant
N	Non-host plant		Unknown

			Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
Family	Species	Cultivar	Aphelenchoides fragariae	Aphelenchoides ritzemaibosii	Aphelenchoides sp	Ditylenchus dipsaci	Meloidogyne arenaria	Meloidogyne chitwoodi	Meloidogyne fallax	Meloidogyne halpa	Meloidogyne incognita	Meloidogyne javanica	Pratylenchus Penetrans	Pratylenchus spp.	Paratylenchus bukowinensis	Longidorus spp.	Xiphinema spp.	Rotulenchulus reniformis	Trichodoridae
Acanthus	spinosissiums									X									
Achillea		'Anthea'					X				X								
Achillea		'Coronation Gold'								N									
Aconitum			X					X	X	X			X						
Aconitum	carmichaelii	'Arendsii'								X									
Aconitum	lycoctonum spp. ranunculifolium									X									
Acorus	calamus											X							
Actea (Cimicifuga)	acerina									X									
Actea (Cimicifuga)	dahurica									X									
Actea (Cimicifuga)	simplex	'White Pearl'								X									
Actea (Cimicifuga)										X			X						
Ajuga	reptans	'Burgundy Glow'								X									
Alchemilla	mollis									X			X						
Allium						X													X
Amaranthus										X									X
Amaranthus	caudatus	UC54						X											
Amaranthus	caudatus	UC57						N											
Amaranthus	hypochondriacus									X									
Amaranthus	retroflexus	UC275						N											
Amaranthus	tinctorius																		
Ammi															X				
Anaphalis			X							X									
Anchusa	azurea	'Dropmore'								X									
Anemone			X							X	X		X						X
Anemone		'Königin Charlotte'										X							
Anemone	coronaria										X								
Anemone	hupehensis var. japonica									X									
Anemone	hupehensis									X									
Anemone	x hybrida				X														
Anethum															X				
Angelica															X				
Antirrhinum							X			X	X	X	X						
Antirrhinum	majus										X		X						

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Antirrhinum	majus	'First Ladies'					X				X	X							
Antirrhinum	majus	'Margaret'					X			X	X	X							
Aquilegia													X						
Aquilegia		'Blue Star'								X									
Arabis	caucasia	'Compinkie'	X							X									
Arctotis	breviscapa												X						
Artemisia	schmidtiana	'Silver Mound'								X									
Artemisia			X																
Artemisia	austriaca									N									
Asclepias										N			X						
Asclepias	tuberosa									N									
Aster			X	X									X			X	X		
Aster	novae-angliae	'Harrington's Pink'								N									
Aster	novae-angliae	'Septemberrubin'								N									
Aster	novi-belgii	'Mount Everest'								N									X
Astilbe													X			X	X		X
Astilbe	(Japonica Groep)	'Peach Blossom'								X									
Astrantia			X							X			X		X				
Astrantia	major	'Rosensinfonie'								X									
Astrantia	major	'Sunningdale Variegated'			X														
Belamcanda	chinensis									N									
Bellis	perennis										X								
Bergenia			X										X						
Boltonia										X									
Boltonia	asteroides	'Pink Beauty'								X									
Bouvardia											X								
Brunnera	macrophylla	'Jack Frost'			X														
Buddleja	spp.												X			X			
Bupleurum															X				
Calendula											X		X						
Calendula	officinalis										X		X						
Callistephus											X	X							
Callistephus	chinensis										X								
Campanula			X							X			X						
Campanula	poscharskyana									X									
Canna	indica										N								
Carum	alpinum									N									
Centaurea			X	X							X		X						
Centaurea	cyanus										X		X						

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Centranthus										X									
Centranthus	ruber	'Albus'								X									
Chelone			X										X						
Chelone	obliqua									N									
Chrysanthemum	coronarium										N								
Chrysanthemum	indicum										N								
Chrysanthemum	morifolium										N								
Chrysanthemum			X							X			X						
Chrysanthemum	carinatum										N								
Clematis		'Hagley Hybrid'						X	X	X									
Convallaria			X										X						
Coreopsis	verticilata																		
Cornus	florida									X									
Dahlia							X/N	X	X										X
Delphinium			X					X		X	X		X						X
Delphinium	ajacis										X								
Delphinium	grandiflorum									X									
Delphinium	grandiflorum	'Blue Mirror'								X									
Dianthus										X	X		X						
Dianthus	barbatus	'Indianer Teppich'								X									
Dianthus	barbatus					X					X		X						
Dianthus	caryophyllus										X								
Dianthus	caryophyllus	'Antalia'									X								
Dianthus	caryophyllus	'Astra'									X								
Dianthus	caryophyllus	'Beta'									X								
Dianthus	caryophyllus	'Carmit'									X								
Dianthus	caryophyllus	'Castelaro'									X								
Dianthus	caryophyllus	'Darling'									X								
Dianthus	caryophyllus	'Desio'									X								
Dianthus	caryophyllus	'Echo'									X								
Dianthus	caryophyllus	'Elegance Korea'									X								
Dianthus	caryophyllus	'Espana'									X								
Dianthus	caryophyllus	'Galil'									X								
Dianthus	caryophyllus	'Imperial White'									X								
Dianthus	caryophyllus	'Izu Pink'									X								
Dianthus	caryophyllus	'Kappa'									N								
Dianthus	caryophyllus	'Lena'									X								
Dianthus	caryophyllus	'Mars'									X								
Dianthus	caryophyllus	'Mercury'									X								

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Dianthus	caryophyllus	'Rachel'									X								
Dianthus	caryophyllus	'Rara'									X								
Dianthus	caryophyllus	'Red Corso'									X								
Dianthus	caryophyllus	'Red Lena'									X								
Dianthus	caryophyllus	'Roland'									X								
Dianthus	caryophyllus	'Rony'									X								
Dianthus	caryophyllus	'Sarinah'									X								
Dianthus	caryophyllus	'Saturnus'									X								
Dianthus	caryophyllus	'Scarlet Elegance'									X								
Dianthus	caryophyllus	'Shinkibo'									X								
Dianthus	caryophyllus	'Target'									X								
Dianthus	caryophyllus	'Tasman'									X								
Dianthus	caryophyllus	'Virgo'									X								
Dianthus	caryophyllus	'White Royalitee'									X								
Dianthus	caryophyllus	'Yellow Dusty'									X								
Dianthus	chinensis	'Baby Doll Mix'					X				X	X							
Dianthus	chinensis	'Princess Scarlet'									X								
Dicentra										X	X	X	X						
Dicentra	spectabilis												X						
Dicentra	spectabilis	'Alba'								X									
Digitalis	ambigua									X	X								
Digitalis	purpurea	Excelsior Hybrids								X									
Digitalis	lantana										X	X							
Digitalis	purpurea										X		X						
Doronicum			X							X			X						
Doronicum	orientale	'Magnificum'								X									
Echinacea	purpurea						N				N								
Echinacea	purpurea	'Leuchtstern'								N									
Echinops										X									
Echinops	bannaticus	'Taplow Blue'								X									
Epimedium										N			X						
Epimedium	versicolor	'Sulphureum'								N									
Eremurus			X										X						
Erica								X											
Erigeron			X	X									X						
Eryngium										X/N					X				
Erysimum	cheiri										X		X						
Erysimum											X		X						
Eschscholtzia	californica										X								

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Filipendula	rubra	'Venusta'								X									
Fragaria	ananassa						N		X		N								
Fritillaria			X																
Gaillardia		'Kobold'								N									
Gaillardia	pulchella	var. picta									N								
Gardenia	jasminoides						X			X	X								
Gardenia	jasminoides	'Radicans'					X												
Gentiana		'Benichidori'								X									
Geranium			X					X		X			X						
Geranium	cinereum	'Laurence Flatman'					X				X								
Geranium	dalmaticum									X									
Geranium	endressi	'Wargrave Pink'								X									
Geranium		'Ann Folkard'					X				X								
Geranium	x magnificum									X									
Geranium	x oxonianum	'Thurstonianum'								X									
Gladiolus		'Oscar'									X								
Gladiolus		'Pink Friendship'									X								
Gladiolus		'Snow Princess'									X								
Gladiolus		'Sylvia'									X								
Gladiolus		'Vinks Glory'									X								
Gladiolus		'White Friendship'									X								
Gladiolus				N				X/N	N	N	X								X
Godetia	grandiflora												X						
Gypsophila										X	X		X						
Gypsophila	elegans	'Covent Garden'									X								
Hedychium	coronarium										X								
Helenium		'Moerheim beauty'								X									
Helenium										N									
Helianthus							X	X		X	X	X	X	X					
Helianthus	annuus						X			X	X	X	X	X				X	
Helichrysum	bracteatum									X	X								
Helictotrichon	sempervirens									X									
Heliopsis	helianthoides	'Karat'								X									
Helleborus	~		X										X						
Hemerocallis								N	X	X/N			X?N						
Hemerocallis	aurantiaca										X								
Hemerocallis		'Marion Vaughn'									X								
Hemerocallis		'Bright Banner'								X/?									
Hemerocallis		'Bright Banner'								X/?									

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			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Heuchera			X										X						
Heuchera	cylindrica	'Green Ivory'					X				X								
Heuchera	sanguinea												X						
Hibiscus											X								
Hibiscus	rosa-sinensis	'Anderson Crepe'									X	X							
Hibiscus	rosa-sinensis	'Delight'									X	N							
Hibiscus	rosa-sinensis	'Fancy Lady'									X	X							
Hibiscus	rosa-sinensis	'Florida Sunset'									X	N							
Hibiscus	rosa-sinensis	'Go-Go Girls'									X	X							
Hibiscus	rosa-sinensis	'Kona'									X								
Hibiscus	rosa-sinensis	'Minerva'									X	X							
Hibiscus	rosa-sinensis	'Old Gold'									X	X							
Hibiscus	rosa-sinensis	'Painted Lady'									X	X							
Hibiscus	rosa-sinensis	'Philipino'									X	X							
Hibiscus	rosa-sinensis	'President'									X	X							
Hibiscus	rosa-sinensis	'Pride of Hankins'									X	X							
Hibiscus	rosa-sinensis	'Rowena Wedding'									X	X							
Hibiscus	rosa-sinensis	'Versicolor Pink'										X							
Hosta			X			X		X		X			X						
Hyacint			N	N		X		N	N		N								
Hydrangea			X							X									
Hydrangea	paniculata	'Grandiflora'								X									
Hypericum										X									
Hypericum	olympicum									X									
Ilex										X									
Ilex	crenata	Tumb.'Hetzii'								N									
Ilex	x	'Nellie R. Stevens'								N									
Ilex	x attenuta	'Sunny Foster'								N									
Impatiens	balsamina										X								
Impatiens	walleriana	'Aztec'					X												
Impatiens	walleriana	'Chickasaw'					X												
Impatiens	walleriana	'Chippewa'					X												
Impatiens	walleriana	'Creek'					X												
Impatiens	walleriana	'Fuchsia'					X												
Impatiens	walleriana	'Futura Red'					X												
Impatiens	walleriana	'Futura White'					X												
Impatiens	walleriana	'Garden Blue'					X												
Impatiens	walleriana	'Hopi'					X												
Impatiens	walleriana	'Maya'					X												

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Impatiens	walleriana	'Navajo'					X												
Impatiens	walleriana	PI 345261					X												
Impatiens	walleriana	PI 345264					X												
Impatiens	walleriana	PI 345265					X												
Impatiens	walleriana	Scarlet					X												
Impatiens	walleriana	'Scarlet Baby'					X												
Impatiens	walleriana	Series F1 (Pink)					X												
Impatiens	walleriana	Series F1 (Rose)					X												
Impatiens	walleriana	Series F1 (Salmon)					X												
Impatiens	walleriana	'Shawnee'					X												
Impatiens	walleriana	'Twinkles'					X												
Iris								X	X	X	X								
Iris	(Germanica Groep)	'Afternoon Delight'								X									
Iris	(Pumila Groep)	'Efin Queen'								X									
Iris	siberica	'Maranantha'								X									
Iris	sibirica	'Blue King'								X									
Iris	sibirica	'Snow Queen'								X									
Iris	tingitana										X								
Justicia	betonica										X								
Kniphofia			X										X						
Kochia	trichophylla										N								
Koelreuteria	paniculata						X			X	X	X							
Lathyrus											X		X						
Lathyrus	latifolis									X									
Lathyrus	odoratus										X		X						
Lavandula	angustifolia	'Munstead Dwarf'								X									
Lavandula			X	X						X									
Lavandula	angustifolia						X				X	X							
Leucanthemum											X								
Leucanthemum	maximum	'Alaska'									X								
Leucanthemum	x superbum	'Exhibition'								N									
Leucanthemum	x superbum	'Polaris'								X									
Liatris			X	X									X						
Liatris	scariosa	'White Spires'								N									
Liatris	spicata									N									
Ligularia			X							X									
Ligularia	dentata	'Desdemona'								X									
Ligularia	stenocephala	'The Rocket'			X														
Lilium	longiflorum		X	X		N		N	N		N		X			X			X

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Limonium			X	X															
Linaria													X						
Linaria	bipartata										N								
Linum	grandiflorum										N								
Liriope										N									
Liriope	muscari	'Evergreen Giant'					X				X	X							
Liriope	muscari	'Variegata'								N									
Lithospermum										N									
Lithospermum	diffusa	'Grace Ward'								N									
Lobelia	cardinalis	'Complement Scarlet'								X									
Lobelia	erinus										X								
Lobularia	maritima										X								
Lupinus										X	X		X						
Lupinus	hartwegii	'Hartweg's Bluebonnet'									X								
Lupinus		Russell Hybrids								X									
Lychnis	spp.												X						
Lysimachia						X				X									
Lysimachia	clethroides									X									
Lythrum	salicaria	'Morden Pink'								X									
Lythrum										X									
Matthiola											X								
Matthiola	incana										X								
Melissa	officinalis									N		X							
Mesembranthemum	tricolor										X								
Miscanthus										X									
Miscanthus	sinensis	'Silberfeder'								X									
Molucella	laevis										N								
Monarda	citriodora						N				N								
Monarda	didyma	'Cambridge Scarlet'								N	N	N							
Musa	sumatrana	'Rowe Red'					X				X	X							
Myosotis										N									
Myosotis	alpestris	'Indigo Blue'								N/?									
Myosotis	sylvatica												X						
Nemesia													X						
Nepeta	nervosa						X				X								
Nigella										X	X		X						X
Nigella	damascena									X	X		X						X
Ocimum	basilicum						X				X	X							
Ocimum	vulgaris									N									

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			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Ophiopogon	japonicus										X								
Origanum							X					X							
Origanum	majorana						N				X/N	N							
Origanum	onites						X				X	X							
Origanum	vulgare						X				X/N	X							
Paeonia			X							X			X/N						
Papaver										X			X						X
Papaver	orientale												X						
Papaver	somniferum									X									
Papaver	dubium										X								
Papaver	orientale	'Carousel'								N									
Papaver	orientale	'Oriental'									X								
Papaver	rhoeas												X						
Penstemon		'Purple Passion'					X				X								
Penstemon							X			X	X								
Penstemon										N									
Penstemon	digitalis	'Husker Red'								X									
Perovskia	alriplicifolia										X								
Petunia							X				X	X							
Philodendron	laciniatum										X								
Philodendron	selloum										X								
Phlox	drummondii										X								
Phlox	(Paniculata Groep)	'Bright Eyes'											X						
Phlox	(Paniculata Groep)	'Eva Cullum'					X				X								
Phlox	(Paniculata Groep)	'Fairest One'								X									
Phlox	(Paniculata Groep)	'Franz Shubert'					X				X								
Phlox	(Paniculata Groep)	'Oakington Blue'					X				X								
Phlox	stolonifera	'Bruce's White'								X									
Phlox			X			X				X			X			X	X		
Photinia	x fraseri									X									
Physalis										X									
Physostegia			X			X				X			X						
Physostegia	virginiana	'Bouquet Rose'								X									
Physostegia	virginiana	'Summer Snow'								X									
Pisum	sativum										X								
Polemonium	reptans	'Firmament'								X									
Polianthes											X								
Polianthes		Single									X								
Polianthes	tuberosa	Double									X								

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Polygonum			X				X			X	X		X						
Potentilla	nepalensis	'Miss Wilmott'	X					X		X									
Primula	japonica	Red Field Hybrids								X									
Primula		Crescendo series								X									
Prunus	cerasifera	'Pissardii'								X									
Prunus	x cistena									X									
Prunus	glandulosa									X									
Prunus	serrulata	'Kanzan'								X									
Prunus	x yedoensis									X									
Pulmonaria			X			X				X			X						
Ranuculus													X						
Ranuculus													X						
Rodgersia	pinnata									X									
Rosa										X	X	X				X			
Rosa	chinensis												X						
Rosa	dumetorum	Laxa								X									
Rosa	indica										X	X							
Rosa	multiflora									X									
Rosa	x odorata									X									
Rosa	rubiginosa									X									
Rosa (onderstam)	canina	'Brogs Stachellose'								X									
Rosa (onderstam)	canina	'Heinsohn's Rekord'								X									
Rosa (onderstam)	canina	Inermis								X									
Rosa (onderstam)	canina	Pfänder								X									
Rosa (onderstam)	canina	'Pollmer'								X									
Rosa (onderstam)	canina	'Schimds Ideal'								X									
Rosa (onderstam)	canina	'Succes'								X									
Rosa (onderstam)		'Manetti'								X									
Rosemarinus	officinalis						X				X	X							
Rudbeckia			X	X						X									
Rudbeckia	fulgida	'Goldsturm'								X									
Rudbeckia	maxima				X														
Rudbeckia	laciniata	'Goldquelle'								X									
Salvia			X				X			X	X		X						
Salvia	azurea	'Grandiflora'								X									
Salvia	haematodes									X									
Salvia	jurisicii									X									
Salvia	leucantha						X				X	X							
Salvia	nemorosa						X				X								

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Salvia	officinalis						X				X	X							
Salvia	splendens	'Bonfire'									X	X							
Salvia	splendens	'Carabiniere Red'									X								
Salvia	splendens	'Flare'									X	X							
Salvia	splendens	'Hotline Red'									X								
Salvia	splendens	'Hotline White'									X								
Salvia	splendens	'Lady in Red'									X	X							
Salvia	splendens	'Oxford Blue'									X	X							
Salvia	splendens	'Rhea'									X								
Salvia	splendens	'Sea Breeze'									X	X							
Salvia	splendens	'Victoria'									X	X							
Salvia	splendens	'Victoria Blue'									X								
Sanguisorba	obtusa									X									
Satureja	hortensis						X				X	X							
Satureja	montana						X				X	X							
Saxifraga			X																
Scabiosa			X							X									
Scabiosa	caucasica	'Fama'								X									
Scabiosa	atropurpurea												X						
Scabiosa	caucasica	Stäfa											X						
Scadoxus	multiflorus spp.	katherinae									X								
Sedum			X										X						
Sidalcea		'Elsie Heugh'								X									
Sidalcea	candida									X									
Sidalcea										X									
Sidalcea		'Partygirl'								X									
Solanum	nigrum										X	X							
Solidago										X/N			X						
Solidago	sphacelata	'Golden Fleece'								X									
Solidaster													X						
Spiraea	japonica	'Froebelii'								X									
Spiraea	x vanhouttei									X									
Stachys	byzantina									X			X						
Stokesia	laevis	'Blue Danube'								X									
Tagetes										X	X					X			X
Tagetes	erecta	'Carnation'								X									
Tagetes	erecta	'Inca Gold'									X								
Tagetes	patula	'Dwarf Primose'					X				X	X							
Tagetes	patula	'Favourite'											X						

Family	Species	Cultivar	Leaf nematodes			Stem nematodes	Root knot nematodes						Root lesion nematodes		Pin nematodes	Virus-transmitting nematodes			
			fragariae	ritzemabosii	sp	dipsaci	arenaria	chitwoodi	fallax	halpa	incognita	javanica	Penetrans	spp.	bukowinensis	spp.	spp.	reniformis	
			Aphelenchoides	Aphelenchoides	Aphelenchoides	Ditylenchus	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Meloidogyne	Pratylenchus	Pratylenchus	Paratylenchus	Longidorus	Xiphinema	Rotulenchulus	Trichodoridae
Tagetes	patula	'Goldie'					X			X	X								
Tagetes	patula	'Petite Gold'					X			X	X								
Tagetes	patula	'Petite Harmony'					X			X	X								
Tagetes	patula	'Tangerine'					X			X	X								
Tanacetum	coccineum	Giant Hybrids								X									
Tanacetum										X									
Tanacetum	cinerariifolium									X									
Tanacetum	parthenium									X									
Teucrium	fruticans									X									
Thalictrum										X									
Thalictrum	flavum ssp. Glaucum									X									
Thymus	praecox	'Albiflorus'								X									
Trachymene															X				
Tradescantia			X							X/N			X						
Tradescantia		'Valour'								X									
Tradescantia		'J.C. Weguelin'								X									
Trollius	~		X							X			X						
Trollius	chinensis	Golden Queen								X									
Trollius		'Lemon Queen'								X									
Verbascum	phoeniceum	Benary's Hybrid								X									
Verbena										X	X								
Verbena	bipinnatifida										X								
Verbena	hybrida										X								
Verbena	officinalis										X								
Verbena	x hybrida	'Florist'					X				X	X							
Verbena	x hybrida	'Showtime Blaze'									X								
Veronica	~						X			X	X		X						
Veronica	longifolia	'Blauriesin'											X						
Veronica	spicata	'Icicle'								X									
Viburnum	carlesii									X									
Vinca	minor	'Bowles variety'								X									
Xeranthemum	annuum									X									X
Zinnia							X				X	X							
Zinnia	elegans	Scarlet					X				X	X							
Zinnia	angustifolia										X