Biology and management of the root-knot nematode *Meloidogyne chitwoodi* in field vegetable crops

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I am not young enough to know everything
-Oscar Wilde-

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BIOLOGY AND MANAGEMENT OF THE ROOT-KNOT NEMATODE
MELOIDOGYNE CHITWOODI IN FIELD VEGETABLE CROPS

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD)
in Applied Biological Sciences (: agricultural sciences)

WIM WESEMAEL

BIOLOGIE EN MANAGEMENT VAN DE WORTELKNOBBELNEMATODE

MELOIDOGYNE CHITWOODI IN DE VOLLEGRONDSGROENTETEELT

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ACKNOWLEDGEMENTS

Working with little animals made me realize how important it is to protect the big mammals of our planet. After many months of looking through a binocular microscope counting root-knot nematodes, the desire to see an elephant becomes extremely high. And actually, in a farmers point of view root-knot nematodes and elephants are not so different; farmers don't want them in their fields. However, economic losses due to elephants are more than compensated by the financial benefits generated through safaris. Maybe we should consider the organisation of nematode safaris. There might be a public amongst elephantologists. And I'm sure that my promotor prof. dr ir Maurice Moens is able to convince people to sign in. Maurice, your enthusiasm is infectious and has made many victims, I'm proud that I'm one of them. Many times I tested your belief in me but you always trusted me and gave me the opportunity and freedom to find my own way. And apart from keeping your door always open, you also opened many other doors for me. This thesis would never have been possible without your help and friendship.

Another enthusiast who helped me around is promotor prof. dr ir Luc Tirry. He took the big risk to recommend me to Maurice. We did not see each other that often, but I enjoyed our meetings where we first talked about racing bikes and afterwards about science. I hope there will come a day that you have a little less work and that you are free of injuries so that we can do a biking trip together.

I would like to thank the members of my jury, prof. dr ir Walter Steurbaut, prof dr ir Erik Van Bockstaele, prof. Thomas Been, prof. Roland Perry, ir Leendert Molendijk and dr ir Raf De Vis for critically evaluating my work.

Leendert, my first contact with root-knot nematode problems was when you took me on a nematode safari 'avant la lettre' in The Netherlands. Your practical approach of nematode problems made a big impression on me and has influenced my work. The talks I had with you and dr Gerard Korthals have helped me to develop my research.

When you take people on a safari they want to see animals. Unlike South African leopards, nematodes don't have a chip to localize them. Luckily I got the help of Thomas Been and his charming wife dr Corrie Schomaker who introduced me into the complex mathematical world of soil sampling and modelling. I could not always keep up with your conversations, but I appreciate your patience and I learned a lot from you. Both of you always encouraged me. Unfortunately I could not convince Thomas to change his preference for the

sugar saturated 'Lindemans kriek' into a true kriek lambic. It is clear that his beer-tasting model can be improved.

Planning to take in-depth soil samples is one thing, collecting them on a monthly basis is a hell of a job. During two successive years I had the help of Etienne De Smet and Hubert Van de Sype. I can not express my gratitude enough to both of you. Not only is an important part of my thesis based on our sampling trips, I also enjoyed our conversations during the many hours we spend on the road. Also Gert De Bondt, Erwin De Poorter and Maarten Vermassen joined me many times to the field.

Erwin showed me the light in the glasshouses and Gert looked after my plants when I was away. On top of that he also massacred millions of white flies and protected my plants against mildew. Meraj Ansari, Jurgen Desamblanx, Tina Mahieu and Hannelore Moens helped me many times with setting up of experiments and cheering up the hours in the lab and the glasshouse.

During a nematode safari it is obvious people want to know what they see. I must confess I would be a very bad guide. Luckily there were a lot of specialised people around to help me. My thanks and respect go to Nancy de Sutter, dr Gerrit Karssen, Hannelore Moens, Marijke Segaert and Lieven Waeyenberge who morphologically and/or molecularly indentified my nematodes when I was in doubt. A special word of thanks I would also like to dedicate to Gerard Hendrickx who developed a fully automated zonal centrifuge which allowed me to safe a lot of time and work in extracting nematodes from soil and plants.

During all these years I've been working at ILVO in Merelbeke. I must say that the last year the conditions were harsh due to never ending construction works. Luckily this burden was easily compensated by the presence of fine colleagues. I want to mention Hans Casteels, dr Lutgard De Wael, dr Kurt Heungens, Liliane Meersschaut, Gerard Meganck, dr Nicole Viaene and Johan Witters but also many others have made my life easier or at least more cheerful. Thank you all.

The most rewarding of my time at ILVO is that some of the colleagues have become friends. Hanneke, your 'klopklop' on my door brought laughter to my heart and I feel privileged I can call myself a regular consumer of the great cakes you and Michael prepare. Steven, it is incredible how much love you spread in this world, your friendship means a lot to me. Eduardo, you witnessed most of my writing process and your presence was inspiring for my scientific work. You let me enjoy writing papers and you were always the first one I bothered when I was facing problems or when I had questions. Our discussions about science, cycling, Belgian politics and the reorganisation of the universe, together with your hilarious

view on Belgian life, coloured the last months of my PhD. Unfortunately bad forces separated us the last weeks you were at ILVO. I whish you a lot of success in your scientific career and I hope we can work together in the future.

When you are on a safari, at night at the camp fires, stories are told and legendary people are often mentioned. It is clear that on a nematode safari a lot of these stories will have prof. dr Roland Perry in the leading role. Rolo, you were always there to help me and to encourage me. Thanks for sharing your knowledge and experience with me. I spend some great moments in your presence and I hope there are many more to come. You even learned me how to appreciate real ale. My beer cellar will always be open for you.

My PhD thesis is part of a research project that is funded by the grower associations BND and Rijke Oogst. I would like to thank their members for supporting my work. I learned a lot from the contacts I had with the farmers. I hope my work will contribute to help avoiding root-knot nematode problems in the future. I thank Paul Roost for supplying fields for experiments. I would like to thank Marc Willocx from La Corbeille for supplying seeds and advice on farmer practices. The same gratitude goes to Rik Ceyssens, Paul Claesen, Jan Ermel and Willy Smeets from Noliko. Rik, your no-nonsense approach made a great impression on me.

I want to thank my friends for life for supporting me in whatever I do. The card playing nights with 'De Wiezers', Karel, Kris and Steven are priceless and Gerlinde, our quests around the globe always bring laughter to my life.

Finally I want to express my love to mom and dad. Both of you allowed me to find my way and never put any pressure on me. I hope I made you proud.

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Chapter 1

General introduction

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Root-knot nematodes are classified within the genus *Meloidogyne* Göldi, 1892 and are found worldwide. They are obligate plant pathogens and parasitize nearly every species of higher plants. In warm climates and glasshouses *M. incognita, M. javanica* and *M. arenaria* are ubiquitous. In temperate agriculture *M. hapla*, *M. chitwoodi* and *M. fallax* are prevalent.

In the Pacific Northwest of the United States severe damage allocated to root-knot nematodes was reported on potatoes (Golden *et al*., 1980). At first, the damaging nematodes were identified as *M. hapla* but further examination in 1977 revealed that they represented an undescribed species of *Meloidogyne*. This new species was described by Golden *et al*. (1980) and named *M. chitwoodi*, the Columbia root-knot nematode. In contrast with *M. hapla*, this new species reproduced on corn (Santo *et al*., 1980), therefore the Dutch name of *M. chitwoodi* became 'maïswortelknobbelaaltje'. In The Netherlands, *M. chitwoodi* was reported in the beginning of the eighties (Molendijk, PPO, Lelystad, pers. comm.). However, re-examination of preserved potato tubers from 1930 showed that these tubers were infected with *M. chitwoodi*, revealing a long presence of this nematode in The Netherlands (Brinkman *et al*., 1996). The detection of *M. chitwoodi* juveniles in soil samples from an oak forest in Belgium, and the relative high genetic distances observed between Belgian populations confirmed its long presence in the Low Countries (Waeyenberge & Moens, 2001). The increasing problems since the 1980s can be explained partly by the decreasing use of chemical soil disinfection for the control of potato cyst nematodes (Molendijk & Mulder, 1996). Before, most likely these practices also kept *Meloidogyne* spp. at bay. The increasing traffic of machinery between farms and the use of green manure crops instead of fallow periods in between crops might have contributed too. In 1992 a field plot in The Netherlands was established to examine the host suitability of different crops for *M. chitwoodi*. Due to conflicting results with previous studies in the US, the population was re-examined and it was concluded that the root-knot nematodes present in the experimental field were both morphologically and biologically different from *M. chitwoodi* (Karssen, 1995). This root-knot nematode was also different from other known nematodes and was described as a new species, *M. fallax* (Karssen, 1996). Both *M. chitwoodi* and *M. fallax* caused severe damage on economically important crops such as potato, black salsify and carrot and, therefore, both species were listed as quarantine pests in the EU in 1998 to avoid their further spread.

Generally *Meloidogyne* spp. can occur on a wide range of soil types, but their association with crop damage is strongly associated with sandy soils or sandy patches

within fields (Van Gundy, 1985). In Belgium severe damage caused by *M. chitwoodi* became prevalent after the 1990s in the sandy soils of the provinces of Antwerp and Limburg in field vegetable crops for the canning industry. Major problems were reported in black salsify (*Scorzonera hispanica* L.) and carrots (*Daucus carota* L.). Quality control of these crops before harvest revealed marked quality damage on the tap roots. Severe galling induced a rough surface rendering infected crops unprocessable. When the percentage of deformed crops becomes too high (> 30%) the harvest of the crop is no longer profitable. Therefore, it became very important to know in advance when fields were infested with *M. chitwoodi*. Soil sampling and diagnostic analysis allowed the canning industry to avoid the production of black salsify and carrot in infested fields. However, the farmers often used these fields to grow ware potatoes, which also suffered from *M. chitwoodi*-induced damage and built up high populations of this pest. The wide host range of *M. chitwoodi* (Santo *et al*., 1980; O'Bannon *et al*., 1982; Ferris *et al*., 1993; den Nijs *et al*., 2004) makes decisions on crop rotations very difficult. Moreover, *M. chitwoodi* is able to reproduce on many weeds (Thomas *et al*., 2005; Kutywayo & Been, 2006) and complete resistant crops are not available.

The use of pesticides seemed to be the only solution. However, in general nematicides depress but do not eliminate populations of plant-parasitic nematodes and, therefore, final nematode densities may be too high for a profitable crop to be grown the following season without further phytosanitary measures being taken (Hague & Gowen, 1987). The high cost of pesticides makes them only profitable on high-value crops.

Crops grown on fields declared free of *M. chitwoodi*, based on sampling results, were sometimes severely damaged. Sampling procedures were based on schemes developed for potato cyst nematodes and new research was required for *M. chitwoodi*. In addition to horizontal distribution, vertical distribution is also important for sampling procedures as migration of *M. chitwoodi* from deeper soil layers was reported by Pinkerton *et al*. (1987).

The aim of this dissertation was to increase the knowledge of the biology of *M. chitwoodi* in relation to field vegetable crops and to develop a strategy to control this soil borne pest. An important part of this work focussed on different aspects that could help to increase the detection chances of *M. chitwoodi*.

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Therefore I studied:

- The influence of root diffusates and host age on the hatching of *M. chitwoodi* and *M. fallax*.

- The vertical distribution of *M. chitwoodi* under different crops and fallow in naturally *M. chitwoodi*-infested fields.

- The host suitability of fodder beet, summer barley, carrot, bean and marigold for *M. chitwoodi* under field conditions.

- The host suitability of different carrot cultivars for *M. chitwoodi* and damage on carrots caused by infection with *M. chitwoodi*.

- The host suitability of different bean cultivars for *M. chitwoodi*, *M. fallax* and *M. hapla* and the development of *M. chitwoodi* inside the roots.

Chapter 2

Meloidogyne spp. *in Europe*

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2.1   Introduction

Root-knot nematodes are sedentary endoparasites. Only eggs, adult males and second-stage juveniles (J2) can be found freely in the soil. After embryogenesis the first moult occurs within the egg giving a J2 that hatches from the egg. Second-stage juveniles primarily penetrate roots directly behind the root cap at the region of cell elongation, but they can also enter at points where lateral roots emerge, penetration sites of other J2 and cut surfaces of roots (Hussey, 1985). They migrate intercellularly towards the zone of root differentiation in the vascular cylinder. The J2 stop migrating when initial giant cells are induced and a feeding site is established. The juveniles start swelling and moult three times in quick succession without any feeding activity. During the last moult, the male undergoes a true metamorphosis to become a long filiform nematode. The female at first retains the same shape as the last juvenile stage but enlarges as it matures and becomes pyriform (de Guiran & Ritter, 1979). Depending on the host plant and the environmental conditions, a female may lay 30-80 eggs per day. The eggs are enclosed in a gelatinous egg matrix that is usually deposited on the surface of the roots. Sometimes they occur within the galls or root tissue (i.e. potato tubers). The duration of the life cycle depends on the host, climatic conditions and nematode species.

In this chapter the occurrence of *Meloidogyne* spp. in Europe is discussed with special attention for the detection, identification and control of these soil borne pests.

2.2   Presence

Out of more than 90 *Meloidogyne* species described, 22 have been found in Europe (table 2.1). In the cooler climates *M. hapla*, *M. naasi*, *M. chitwoodi*, *M. fallax* and *M. minor* are the most important species; in warmer conditions *M. arenaria*, *M. javanica* and *M. incognita* are prevalent. Most species were described from agricultural areas except for *M. ardenensis*, *M. kralli*, *M. maritima*, *M. duytsi* and *M. ulmi*. *Meloidogyne minor* was detected in coastal dunes in the UK (Fleming *et al*., 2006), which are most likely its natural habitat (Karssen, 2004). Waeyenberge and Moens (2001) isolated *M. chitwoodi* juveniles from oak forest soil indicating a long presence of this root-knot nematode in Belgium, which was confirmed by relatively high genetic distances they observed between Belgian populations. Very few reports on the presence of *Meloidogyne* spp. in natural habitats are available, although this could give valuable information about the origin of the different species and their position in the soil-food web.

Table 2.1: *Meloidogyne* species found in Europe (Karssen & Van Hoenselaar, 1998; Palmisano & Ambrogioni, 2000; Holgado *et al*., 2001; Blok *et al*., 2002; Karssen, 2002; Karssen & Grunder, 2002; Karssen *et al*., 2004; Sirca *et al*., 2004; Vovlas *et al*., 2004; Viaene *et al*., 2007)

Meloidogyne species	Year of description	Host plants	Occurrence
M. javanica	1885	Large host range	General
M. arenaria	1889	Large host range	General
M. exigua	1892	Tomato, Peach, *Bougainvillea glabra*	Greece, Italy
M. incognita	1919	Large host range	General
M. hapla	1949	Large host range	General
M. artiellia	1961	Brassicaceae, Fabaceae, Poaceae	France, Greece, Italy, Spain, United Kingdom
M. graminis	1964	Grasses, cereals	Germany, The Netherlands
M. naasi	1965	Mainly grasses and cereals, dicotyledons	General
M. kirjanovae	1965	Tomato	Russia
M. ardenensis	1968	Trees, shrubs, dicotyledon weeds	Belgium, France, Germany, Norway, Poland, The Netherlands, Russia, Slovakia, United Kingdom
M. ethiopica	1968	Tomato	Slovenia
M. chitwoodi	1980	Large host range	Belgium, France, Germany, The Netherlands, Portugal, Switzerland
M. kralli	1983	Cyperaceae, grasses and cereals	Estonia, Poland, Russia, Switzerland, United Kingdom
M. hispanica	1986	*Prunus persica*, sugarbeet, tomato	France, Portugal, Spain, The Netherlands
M. maritima	1987	Beach grasses	Belgium, France, Germany, The Netherlands, United Kingdom
M. mayaguensis	1988	Tomato	France
M. lusitanica	1991	Olive	Portugal
M. fallax	1996	Large host range	Belgium, France, Germany, The Netherlands, Switzerland
M. duytsi	1998	Beach grasses	Coasts of Western Europe
M. ulmi	2001	Elm	Italy, The Netherlands (?)[*]
M. baetica	2003	Olive, lentisk, *Aristolochia baetica*	Spain
M. minor	2004	Grasses, potato, tomato	Belgium, Ireland, The Netherlands, United Kingdom

[*] The elm trees on which *M. ulmi* was detected in Italy were imported from Wageningen, The Netherlands.

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2.3   Impact

Although *Meloidogyne* spp. are worldwide considered as the most important genera of plant-parasitic nematodes (Sasser & Freckman, 1987), information in scientific literature on the economic impact of root-knot nematodes in Europe is scarce. *Meloidogyne* spp. can seriously affect both quantity and quality of crop production. Severe galling on potato tubers caused by *M. chitwoodi* and *M. fallax*, forking and hairiness of carrots due to *M. hapla* infection, or the yellow patch disease induced by *M. minor* in golf courses are only a few examples of clearly visible effects of root-knot nematodes. However, damage caused by root-knot nematodes is often overlooked. In many cases no above-ground symptoms are observed. Root galling can be limited and galls can be very small (Karssen, 2002).

Apart from direct losses due to nematode attacks, many indirect losses are reported. Indirect losses include waste of irrigation water and fertilizers. Nematode-damaged roots do not utilize water and fertilizers as healthy roots do (Mai, 1985). Disease complexes due to interactions with other nematodes and other pests can occur. Interactions between *Meloidogyne* spp. and *Fusarium* wilt have been reported in many host crops (Abawi & Barker, 1984; Griffin, 1986; France & Abawi, 1994; Siddiqui & Mahmood, 1999). Sclerotia of *Rhizoctonia solani* were found on tomato roots with galls of *M. incognita*, whereas ungalled regions of the roots did not contain sclerotia (Golden & Van Gundy, 1975). Nematode attacks sometimes lower the resistance of plants to diseases caused by other organisms (Mai, 1985).

Economic losses due to *Meloidogyne* spp. do not stop or start with yield reduction. Crop rotations with cash crops can be seriously hampered and infected fields or glasshouses need to be sanitized. Since the end of the previous century preventive soil sampling is conducted in Belgium and The Netherlands to detect *M. chitwoodi* and *M. fallax*. However, the extra costs for the sampling and diagnostic analysis are most likely compensated by the reduction of heavily infested and valueless crops. In Belgium no contract is given to farmers for growing carrots or black salsify for the canning industry if fields are infected with *M. chitwoodi* or *M. fallax*. Both species are listed as quarantine organisms in the EU (EC Directive 2000/29/EC) and EPPO (A2 list n° 6.1 and 6.2). Phytosanitary measures include the control of propagation material (e.g. seed potatoes, flower bulbs).

Estimates on monetary losses due to root-knot nematodes are complex and most likely underestimations.

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2.4 Identification

Traditionally, *Meloidogyne* spp. are described and identified based on their morphology and morphometrics. For reliable identification the best approach is to integrate morphological, isozyme and DNA data, together with information on mode of reproduction, chromosome number, host plants and distribution (Karssen & Moens, 2006). Morphological identification of root-knot nematodes is difficult and time consuming because of overlapping characters (Jepson, 1987; Karssen, 2002). Therefore, many research groups have been developing molecular techniques for their identification.

Protein electrophoresis was the first molecular technique to be applied in nematology (Subbotin & Moens, 2006). Isozyme phenotypes of adult females, especially of esterase and malate dehydrogenase, are considered to be very useful as reliable markers for identification of *Meloidogyne* spp. (Esbenshade & Triantaphyllou, 1985, 1990; Venkatachari *et al.*, 1991; Karssen *et al.*, 1995). Two-dimensional gel electrophoresis, which provided a better protein separation and fingerprint, were used to distinguish *M. chitwoodi*, *M. fallax* and *M. hapla* (van der Beek *et al.*, 1997; Tastet *et al.*, 1999).

Electrophoresis requires adult females, whereas DNA-profiles can be obtained from a few or even single nematodes or eggs. This made the polymerase chain reaction (PCR) - technique the most widely used technique for studying the genetic diversity of root-knot nematodes and their identification. Multiplex PCR methods allow the detection of one or more species in a nematode mixture by a single PCR-test. Zijlstra (1997) identified single juveniles or isolates of *M. chitwoodi*, *M. fallax*, *M. hapla* and *M. incognita* in a single PCR reaction and it was possible to detect species present in mixtures in proportions as low as 2 to 5%. Meng *et al.* (2004) identified single J2s of *M. incognita*, *M. javanica* and *M. arenaria* with specific designed SCAR primers. Recently, Adam *et al.* (2007) unified published PCR-methods and developed a molecular diagnostic key for *M. incognita*, *M. javanica*, *M. arenaria*, *M. mayaguensis*, *M. hapla*, *M. chitwoodi* and *M. fallax* that can be used with single juvenile or adult nematodes.

Real time PCR methods allow simultaneous detection and quantification of several nematode species in one sample. Zijlstra and Van Hoof (2006) developed a multiplex real-time PCR for the simultaneous detection of *M. chitwoodi* and *M. fallax*. The technique proved to be at least 10 times more sensitive than comparable regular PCR techniques also targeting the ITS sequence and allowed precise quantification when only one of the two species was present. However, the ability of the multiplex real time PCR to detect small

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quantities of DNA of one species was reduced when large quantities of DNA of the other species were present.

2.5   Management and control

Management has the objective of minimizing economic losses, and includes the whole system of care and treatment of crop pests, whilst control refers to specific acts designed to reduce the numbers of nematodes (Hooper & Evans, 1993).

2.5.1   Prevention

Avoiding *Meloidogyne* infestations is not obvious. Root-knot nematodes are not found in seeds but may be present in vegetative planting material such as corms, bulbs or roots (Karssen & Moens, 2006). Intensified international trade increases the risk of further spreading of indigenous species or introduction of new species.

The quarantine status of *M. chitwoodi* and *M. fallax* implies that plants and plant products have to be free from these nematodes before they are allowed to enter EU traffic (Anonymous, 2000). The detection is based on visual inspection for symptoms of each product that might form a potential pathway. However, as stated earlier, symptoms are not always visible. For the detection of *M. chitwoodi* on seed potatoes it is suggested that the tubers should be peeled before the extraction process to increase the chances of detection (Viaene *et al*., 2007). In The Netherlands the import and export of potatoes is visually checked for the presence of *M. chitwoodi* and *M. fallax*. If contamination is detected the phytosanitary certificate is refused and the product has to be cleaned, if possible, or destroyed. Infected fields will lose their registration and all propagation material from these fields will be checked during the successive 3 years. Extra inspection will be conducted in known infected areas (den Nijs, 2003). A similar policy is followed in Belgium. Unfortunately, no regulation is present for other *Meloidogyne* species.

Flower bulbs are traded without roots but *M. chitwoodi* and *M. fallax* were found in the bulbs of cultivars of *Chionodoxa*, *Dahlia*, *Gladiolus*, *Hyacinthus*, *Iris*, *Lilium*, *Puschkinia* and *Tulipa* (den Nijs *et al*., 2004) and special attention should be given to their potential as nematode distributors.

A main potential risk is the spreading of nematodes through soil and root fragments adhering to machinery. However, cleaning of machinery is generally not practised.

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Prevention avoids damage and huge population build ups in infected soil. Therefore, damage models like the Seinhorst model can be used to estimate the possible yield losses based on the initial population density (Pi) present in the field at planting date. Different models that can be used for making management decisions are discussed by Ferris and Noling (1987) and McSorley and Phillips (1993). Data on the tolerance limit and the minimum yield of different *Meloidogyne*-plant combinations are given by Schomaker and Been (2006). However, predictions of potential crop losses due to nematodes, based on estimates of nematode population densities, are often not possible due to the interaction between plant-parasitic nematodes and their environment, including other pest species (Noling, 1987). To improve the models intensive soil sampling is required, which is costly and not economically justified.

Preventive soil sampling can help in making decisions on crop rotations. Whereas in the past in The Netherlands 7% of the harvest was rejected for the canning industry, in 2003 this was only 1.5% (Molendijk, PPO, Lelystad, pers. comm.).

2.5.2   Cultural management

In crop rotations susceptible crops are rotated with immune or resistant (see 2.5.5) crops. Possible crop rotations for the control of root-knot nematodes are limited due to the wide host range of some species. Grasses have been effective in reducing populations of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* (Netscher & Taylor, 1979). Barley can be used in rotations to reduce *M. hapla* infections (Bélair, 1996). den Nijs *et al.* (2004) gave an overview on the host status of various crops for *M. chitwoodi* and *M. fallax* resulting in very few options for crop rotations. Marigolds have proved successful against *Meloidogyne* spp., both in glasshouse and field conditions (Ploeg, 1999; Ljani *et al.*, 2000). Their effect against *Pratylenchus* spp. (Pudasaini *et al.*, 2006) makes them an important option if both nematode genera are present.

The population of root-knot nematodes decreases markedly during winter and under fallow (Pinkerton *et al.*, 1991; Noling & Becker, 1994). However, European policy no longer supports fallow periods.

Many weeds are host for *Meloidogyne* (Thomas *et al.*, 2005; Kutywayo & Been, 2006); therefore, adequate weed control is required in crop rotations and fallow periods.

A major limitation to control nematodes by disrupting the continuity of food resources is that this strategy does not fit some intensive agricultural practices and farmers prefer to grow crops that are more economically rewarding (van der Putten *et al.*, 2006). Crop

rotations are often historically inherited and new crops require major investments in machinery and cultural practices. Also the absence of a market for the new crops can limit the introduction of new crop rotations.

Manipulating planting or harvest dates can reduce damage caused by nematodes (Hooper & Evans, 1993), but is not generally practised as planting and harvest depend strongly on climatological conditions and the market demands.

Biofumigation is the action of volatile substances produced in the bio-decomposition of organic matter for plant pathogen control (Bello *et al.*, 2000). The use of organic amendments has a positive influence on the soil physical structure and its water holding capacity, and stimulates the activity of soil biota that produce or improve nematode suppressiveness. Biofumigation with the use of goat, sheep and cow manure, and remains from rice, mushroom, olive, brassica crops and garden wastes has been successful in a large number of crops throughout Spain (Bello *et al.*, 2004). Brassicaceous amendments reduced the survival of *M. javanica* regardless of the glucosinolate concentration of the amendment material (Zasada & Ferris, 2004). Hallman *et al.* (1999) demonstrated the long-term *M. incognita* suppressiveness of chitin amended soil, attributed solely to an increase of fungal and bacterial populations. However, organic amendments must be applied at high rates in order to have a significant effect on nematode populations (Akhtar & Malik, 2000; Zasada & Ferris, 2004; van der Putten *et al.*, 2006). Local resources are recommended for use as biofumigants since the principal limiting factor is the cost of transporting organic matter (Bello *et al.*, 2004).

2.5.3 Physical control

Heat treatments of planting material (e.g. bulbs) can be an important tool to avoid spreading of nematodes. Steaming of soil is expensive and usually only applied in glasshouses for high value crops and for compost. It is not always effective due to the spreading of nematodes in deeper soil layers (Karssen & Moens, 2006) and, therefore, is generally only effective in shallow soils. Soil solarisation requires longer periods of bright sunshine and is only adaptable to regions where sufficient solar energy is available for long periods of time (Mediterranean countries). In Southern Spain, solarisation can be used for the control of *M. incognita* in olive nurseries (Nico *et al.*, 2003). Ioannou (2000) demonstrated that soil solarisation can be an effective alternative for the use of methyl bromide in glasshouses in Cyprus.

2.5.4 Biological control

Nematophagous fungi and bacteria have been the subject of many studies on nematode control (Kerry, 1987). Kiewnick and Sikora (2006) demonstrated that a single pre-plant application of the fungus *Paecilomyces lilacinus* strain 251 could control *M. incognita* on tomato. This fungus is commercialized in Germany, applied as dispersible granules for application in water. Another fungus, *Pochonia chlamydosporia*, provided control of root-knot nematodes on vegetable crops in tropical soils, but results in Europe have been less satisfactionary (Tzortzakakis & Petsas, 2003; Viaene *et al.*, 2006). However, a one-time application of *P. chlamydosporia* was able to slow down the build-up of *M. javanica* for at least 5-7 months in tomato and lettuce rotations in a glasshouse (Van Damme *et al.*, 2005).

Arbuscular mycorrhizal fungi (AMF) are endophytic fungi that grow within plant tissues without causing disease and can play a protective role against parasitic nematodes. Establishment of AMF in olive plants significantly reduced severity of root galling as well as reproduction of *M. incognita* and *M. javanica* (Castillo *et al.*, 2006).

Pasteuria penetrans is a bacterial parasite of root-knot nematodes and can reduce their numbers significantly in some cropping systems (Trudgill *et al.*, 2000). The effectiveness of *P. penetrans* strongly depends on the endospore concentrations and is manifest at the level of root penetration by J2 and the loss of nematode fecundity (Kariuki *et al.*, 2006). However, the high multiplication of root-knot nematodes on many vegetables does not allow the *P. penetrans* population to keep up numerically with host (nematode) abundance (van der Putten *et al.*, 2006).

Biological control agents will generally provide too little control to be effective alone and their successful use in sustainable management strategies will depend on their integration with other control measures (Viaene *et al.*, 2006).

2.5.5 Resistance

Plant resistance is probably the most environmentally safe method to control root-knot nematodes. Resistance against *Meloidogyne* spp. has been reported in many food crops (Cook & Starr, 2006) but it is not often used. The most important example is the resistance against *M. arenaria*, *M. incognita* and *M. javanica* in Mi-gene bearing tomato cultivars which are widely used. However, resistant breaking populations of *M. incognita* and *M. javanica* have been reported in Greece and Spain (Ornat *et al.*, 2001; Tzortzakakis *et al.*, 2005) and this might reduce current use. Resistance against *M. arenaria*, *M. incognita* and *M. javanica* was reported in prunus rootstocks in France and Spain (Fernandez *et al.*, 1994;

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Pinochet *et al*., 1996). Resistance against *M. javanica* was also found in peach and plum rootstocks from Spain, France and Italy (Pinochet *et al*., 1999). Several Me resistance genes against *M. arenaria*, *M. incognita* and *M. javanica* were found in pepper (Djian-Caporalino *et al*., 2007). Resistance for *M. hapla* (Chen & Roberts, 2003) and *M. naasi* (Cook *et al*., 1999) was reported in common bean and ryegrasses, respectively. Promising results have been obtained from several wild tuber-bearing *Solanum* species for resistance against *M. chitwoodi*, *M. hapla* and *M. fallax* (Janssen *et al*., 1996; Brown *et al*., 2006).

2.5.6 Chemical control

The increasing concern about pesticide residues in the food chain, risks to human health and the adverse impact on the environment has reduced the use of nematicides and resulted in the ban of methyl bromide. Nevertheless, approximately 48,000 t active substances are used annually in Western Europe (Haydock *et al*., 2006). Nematicides are reliable and fast working and can give good economic returns on high-value crops. They may be essential for producing nematode-free export crops. However, in general nematicides depress but do not eliminate populations of plant-parasitic nematodes and therefore final nematode densities may be too high for a profitable crop to be grown the following season without further phytosanitary measures being taken (Hague & Gowen, 1987).

Chapter 3

General materials and methods

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3.1 Soil sterilization

The soil used in the culture of *Meloidogyne chitwoodi* and *M. fallax*, the pot experiments, and screening tests, was a sandy soil (sand 87%, loam 9%, clay 4%) collected in Nederweert, The Netherlands. The soil was sterilized with a Sterilo 7K/A soil pasteurizer at 100°C for 12 h (Harter Elektrotechnik, Schenkenzell, Germany). After sterilizing, the soil was stored in a polyethylene container (120x100x76 cm). Six days after sterilizing the soil was ready to use (Harter Elektrotechnik, operating manual).

Meloidogyne chitwoodi or *M. fallax* infected soil, which remained after experiments or breaking up pot cultures, was sterilized before removal.

3.2 Culture of *Meloidogyne chitwoodi* and *M. fallax*

A population of *M. chitwoodi* and *M. fallax*, both from The Netherlands, were maintained as stock cultures on tomato plants, *Lycopersicon esculentum* L. cv. Moneymaker, in 17-cm-diam. plastic pots filled with sterilized soil (see 3.1), in a temperature-controlled glasshouse (20-26°C) with daily 14 h light period. Tomato was used because of the ease of culture and it is an annual crop that is an excellent host for both *M. chitwoodi* and *M. fallax*.

Four to six months after nematodes were added; the infected roots of the tomato plants were washed and put on Baermann funnels (see 3.3.1). Freshly hatched second-stage juveniles (J2) were used as inoculum for young tomato plants to maintain the culture.

3.3 Extraction of nematodes

3.3.1 Baermann funnel

The extraction of nematodes with the Baermann funnel technique (Hooper, 1986) is based on the motility of nematodes and enables them to be separated from soil or organic material.

Roots were cut into small fragments which were put on a filter paper (Ederol Rundfilter, 40 g/m^2, Munktell Filter AB, Falun, Sweden) that was lined in a sieve (mesh 2 mm). The sieve was put on top of a glass funnel filled with tap water to a level that just covered the bottom of the sieve and the plant tissue. The stem of the funnel was connected with a rubber tube that was closed with a clip (Fig 3.1). Second stage juveniles that hatched

from egg masses moved through the filter paper and sieve and accumulated at the bottom of the rubber tube. Nematodes were collected by opening the clip for a few seconds. On a daily basis the water level of the funnels was adjusted to compensate for the evaporation. The water in the funnels was replaced weekly after washing the funnels.
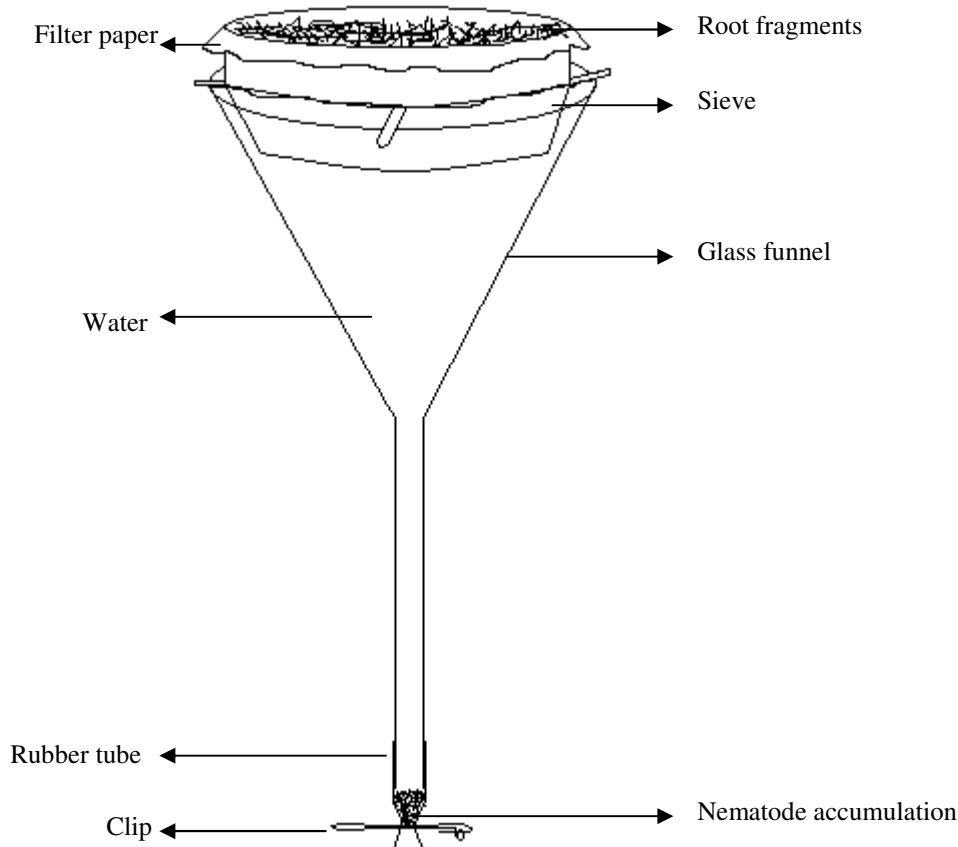
Figure 3.1: Baermann funnel

3.3.2   Zonal centrifuge
3.3.2.1 Sample preparation

Soil sample were thoroughly mixed before 200 g sub-samples were taken. Roots were separated from the mineral soil fraction by washing the sub-sample through an 850-µm-sieve; the mineral soil fraction that passed through the sieve was collected in a 1 l beaker. Roots and soil particles that were retained on the sieve were washed from the sieve into a beaker and stirred. After 3 s without stirring, during which the soil particles settled to the

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bottom, the suspension was poured onto a 250-µm-sieve so that the roots, but not the soil particles, were collected. If present, stems and leaves were removed. The root fraction was blotted, weighed and then macerated for 1 min at high speed with a commercial Waring blender.

3.3.2.2 Centrifugation

Nematodes were extracted from both the organic and mineral soil fraction with an automated zonal centrifugal machine (Hendrickx, 1995). This machine follows the principles of conventional centrifugation but the process is fully automated.

Nematode suspensions of 1l, either with the mineral soil fraction, the macerated organic fraction, or both were sub sampled (500 ml) and automatically transferred along with water and $MgSO_4$ (density = 1.20) into a rotor. In this rotor nematodes were separated from the other components. Nematodes were retained at the interface between the water and the $MgSO_4$ solution. Near the end of the centrifugation process a kaolin suspension was added automatically to the rotor to avoid soil particles and other debris mixing with the nematode suspension when the centrifugation stopped. After centrifugation the nematodes were collected in a glass beaker through the hollow shaft of the rotor. Figure 3.2 gives a schematic view of the rotor and the centrifugal machine.
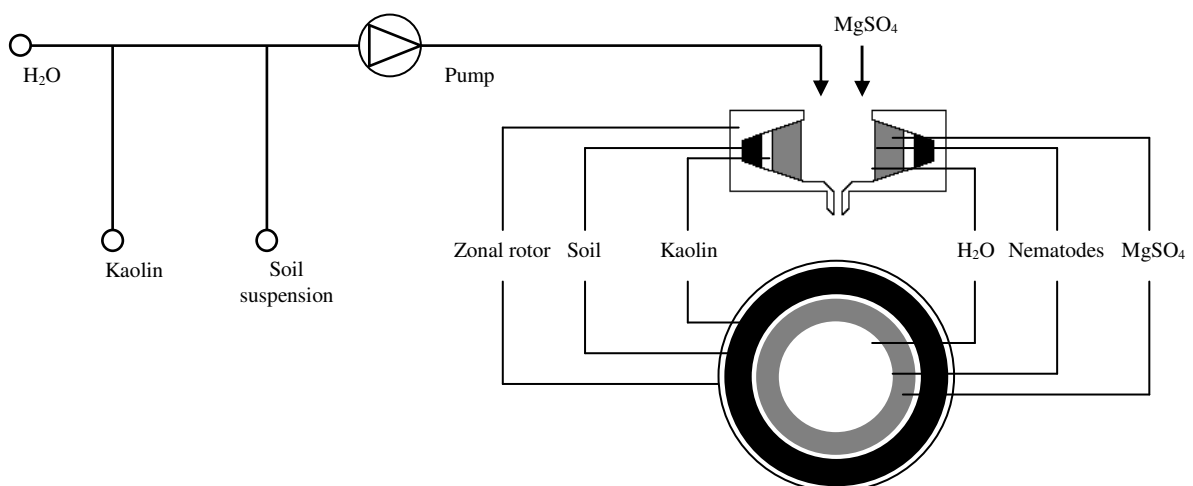
Figure 3.2: Schematic view of the zonal centrifugation machine.

3.4 Screening

3.4.1 Culture of plants

To determine the potential of different plants and cultivars as a host for *M. chitwoodi* and *M. fallax* 40 plants per plant or cultivar were grown individually in plastic folding tubes (15 × 20 × 120 mm). The tubes were filled with sterilized soil (see 3.1) and in each tube one seed was sown. The plants were kept in a temperature-controlled glasshouse (20-26°C) with daily 14 h light period. Plants were watered with an atomizer. Three and six weeks after sowing, the plants were fertilized with a liquid fertilizer (NPK 7-4-6, 5 ml/l, Bayer).

3.4.2 Inoculation

Freshly hatched (< 24h) J2 of *M. chitwoodi* or *M. fallax* were obtained from stock cultures (see 3.2) and collected with the Baermann funnel technique (see 3.3.1). The nematodes were counted with the use of a binocular microscope to determine the density. Dilutions at the appropriate nematode density were made. Each plant was inoculated with 200 J2 one week after emergence. Therefore, a 1 cm deep hole was made in each tube. The nematode solution was transferred into these holes with a micropipette.

3.4.3 Scoring

Eight weeks after inoculation the tubes with the plants were soaked in water and the soil was gently washed away. Subsequently the roots were submerged in a solution of Phloxine B (0.15 g/l tap water) for 15-20 minutes to stain the gelatinous egg sacs produced by the female root-knot nematodes on the roots (Daykin & Hussey, 1985). After staining, root systems were rinsed in tap water to remove residual stain on the roots. The number of egg masses per plant was determined with the use of a binocular microscope.

Chapter 4

The influence of root diffusate and host age on hatching of
Meloidogyne chitwoodi *and* M. fallax[†]

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4.1 Introduction

Survival of root-knot and cyst nematodes between host crops depends to some extent on the hatching responses and changes in the physiology of the unhatched juveniles (Perry, 2002). A survival attribute of many species is the ability to remain dormant during adverse conditions. Dormancy can be separated into quiescence, when the nematodes readily respond to resumption of favourable conditions, and diapause, where there is a time delay before the nematode responds to the return of favourable conditions. In contrast to some cyst nematode species, for which stimulation by host root diffusates is required for substantial hatch (Perry, 2002), most species of *Meloidogyne* hatch in water (de Guiran & Ritter, 1979), although root diffusates can enhance the rate of hatching. Some *Meloidogyne* species are able to survive adverse conditions, and diapause and changes in hatching physiology are important mechanisms that aid survival. In the temperate species, *M. naasi* and *M. hapla*, thermal regimes play an important role in the regulation of diapause (Antoniou & Evans, 1987; Lahtinen *et al.*, 1989). Gaur *et al.* (2000) described the influence of host age on the hatching of juveniles of *M. triticoryzae*. This species has multiple generations during a host growing season and three types of eggs are produced, those that hatch in water, those that hatch in host (rice) root diffusate and those that do not hatch even in the presence of diffusate. The proportion of these three types varies with generation. Egg masses of the final generation, collected from senescing plants, contained a large proportion of juveniles that did not hatch even in the presence of rice root diffusates and were in diapause. Similarly, in some species of cyst nematodes there is a change between generations in the dependence of encysted eggs on root diffusates (Perry, 2002). For example, females of *Heterodera sacchari* developed into cysts that contained approximately 20% more unhatched J2 that were refractory to hatch stimuli and 10-15% that depended on host root diffusate for hatch stimulation, compared to cysts produced on younger plants (Ibrahim *et al.*, 1993). The dependence on root diffusates is a type of obligate quiescence, as defined by Evans (1987).

Studies on the biology of *M. chitwoodi* and *M. fallax*, and host-parasite interactions are important in order to develop an efficient strategy to detect and control these species. This chapter reports the results of comparative studies on the effects of root diffusates and host age on the *in vitro* hatching of both species.

4.2 Materials and methods

4.2.1   Collecting of root diffusates

To obtain host root diffusate, pots containing 6-week-old tomato plants were saturated with distilled water, after which root diffusate was collected (Fenwick, 1949) by pouring approximately 200 ml of distilled water into each pot and collecting the leachate. The root diffusate from eight pots was pooled. Pilot tests showed that diffusate from 6-week-old tomato plants elicited maximum hatch. Soil leachate was collected from pots containing soil only. The root diffusate and soil leachate were filtered (Ederol Rundfilter, 40 g/m^2, Munktell Filter AB, Falun, Sweden). The root diffusate was diluted with distilled water to obtain 50% and 10% concentrations. During the experiment the stocks of root diffusate, dilutions, soil leachate and distilled water were stored in sealed plastic bottles at 4°C.

4.2.2   Collecting of egg masses

To obtain egg masses for hatching tests, ten pots (17 cm diam.) with 6-week-old tomato plants were inoculated with approximately 3000 freshly hatched (< 24 h) J2 of either *M. chitwoodi* or *M. fallax* obtained from the stock culture (see 3.2). Egg masses were collected from 13- and 30-week-old tomato plants, 7 and 24 weeks after inoculation, respectively. The age of the tomato plants was chosen in relation to the life cycle of tomato under the glasshouse growing regime. Plants at the age of 13 weeks were vegetatively fully developed and started flowering. Plants at the age of 30 weeks were starting to senesce. The tomato roots were washed to remove the soil and the infected root parts were separated from the uninfected root parts. It was not feasible to remove intact egg masses from the roots, so small pieces (approximately 5 mm in length) of root, containing a female plus egg mass, were collected for the hatching tests.

4.2.3   Hatching tests

Twenty root pieces containing one female and one egg mass each were put on a 48-μm-sieve and covered with 2 ml of the test solution. The test solutions were 100%, 50% and 10% tomato root diffusate (RD, 0.5RD and 0.1RD, respectively), soil leachate (SL) and distilled water (DW). The sieves were kept in small plastic bottles and covered with a perforated lid. The bottles with the egg masses were stored, fully randomized, in an incubator at 22 ± 1°C. Counts of the hatched J2 and replacement of the test solutions were done at weekly intervals. The experiment was terminated after 12 weeks for *M. chitwoodi*

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and 14 weeks for *M. fallax* when the hatch per week had declined to < 20 J2. The remaining eggs were covered with 10% sodium hypochlorite and homogenised; the numbers of unhatched eggs were counted to determine the percentage hatch. The tests for *M. chitwoodi* 13 weeks RD, 0.5RD, SL, DW and 30 weeks RD, DW and the tests for *M. fallax* 13 weeks RD, 0.5RD, 0.1RD, SL and DW were replicated four times. The tests for *M. chitwoodi* 13 weeks 0.1RD and 30 weeks 0.5RD, 0.1RD and SL and the tests for *M. fallax* 30 weeks RD, 0.5RD, 0.1RD, SL and DW were replicated three times.

4.2.4   Statistical analysis

For the statistical analyses Statistica 5.5 was used. The hatching data obtained for both *M. chitwoodi* and *M. fallax* were fitted to the logistic model $y = c / (1 + \exp(-b*(time - m)))$, where y is the cumulative % hatch; the model is described by three parameters: the time at which 50% hatch is reached (m), the hatching rate (b) and the final hatching percentage (c) (Oude Voshaar, 1994). These parameters were calculated for all the replicates of the treatments separately and subjected to analysis of variance (ANOVA). Results are reported as significant or non-significant in Tukey honest significant difference tests ($P < 0.05$). The effect of host age and the difference between *M. chitwoodi* and *M. fallax* were examined with paired t-tests ($P < 0.05$).

4.3 Results

4.3.1   Effect of root diffusate on hatch

There was no effect of root diffusates on the hatching of *M. chitwoodi* from eggs collected from young (13 weeks) actively growing plants (Table 4.1; Fig. 4.1). In all treatments, the final hatching (c) reached 93 to 95% and the hatching profile during the tests was similar for all treatments. By contrast, eggs collected from old, senescing plants (30 weeks) gave a substantially lower percentage hatch in distilled water (Table 4.1; Fig.4.1). The presence of root diffusates induced a significant increase in the number of hatched J2 with a maximum of 89.8% in the 100% root diffusate treatment. There were no significant differences in parameters m and b.

Table 4.1: Parameters of the logistic curve y = c/ (1 + exp (-b × (time – m))) describing hatching of second stage juveniles from egg masses of *Meloidogyne chitwoodi* collected from 13 week and 30 week old tomato plants in the presence of tomato root diffusate (RD) soil leachate (SL) and distilled water (DW) and the R^2 values. Means ± the standard deviation of the time at which 50% hatching is reached (m), the hatching rate (b) and the maximum hatching percentage (c). Significant differences between host age are marked with ∗, †, ‡ (paired t-test, P < 0.05), significant differences between treatments are marked with a different letter (Tukey HSD, P < 0.05).

	m		b		c		R^2	
	13 wk	30 wk	13 wk	30 wk	13 wk	30 wk	13 wk	30 wk
RD	5.3 ± 1.31	3.2 ± 0.63*	1.4 ± 0.87	1.6 ± 0.33	94.9 ± 2.18	89.8 ± 5.13 a	0.94	0.99
0,5 RD	5.6 ± 0.41	3.5 ± 0.51*	1.1 ± 0.06	1.6 ± 0.35†	95.9 ± 0.50	79.2 ± 1.26‡ab	0.99	0.97
0,1 RD	5.1 ± 0.50	3.9 ± 0.53*	1.5 ± 0.97	1.2 ± 0.23	94.8 ± 1.58	70.2 ± 5.96‡bc	0.96	0.99
SL	4.9 ± 0.96	3.4 ± 1.11	1.8 ± 1.07	1.4 ± 0.12	95.2 ± 1.43	63.3 ± 6.67‡cd	0.97	0.99
DW	5.0 ± 0.89	3.7 ± 0.96	1.2 ± 0.27	1.4 ± 0.59	93.6 ± 3.70	51.2 ± 6.95‡ d	0.99	0.97

Table 4.2: Parameters of the logistic curve y = c/ (1 + exp (-b × (time – m))) describing hatching of second stage juveniles from egg masses of *Meloidogyne fallax* collected from 13 week and 30 week old tomato plants in the presence of tomato root diffusate (RD) soil leachate (SL) and distilled water (DW) and the R^2 values. Means ± the standard deviation of the time at which 50% hatching is reached (m), the hatching rate (b) and the maximum hatching percentage (c). Significant differences between host age are marked with ∗, †, ‡ (paired t-test, P < 0.05), significant differences between treatments are marked with a different letter (Tukey HSD, P < 0.05).

	m		b		c		R^2	
	13 wk	30 wk	13 wk	30 wk	13 wk	30 wk	13 wk	30 wk
RD	3.7 ± 0.16	2.3 ± 0.40*	1.5 ± 0.18	1.4 ± 0.26	90.9 ± 1.31 a	90.5 ± 3.77	0.99	0.99
0.5 RD	4.0 ± 0.13	3.1 ± 1.37	1.5 ± 0.27	1.1 ± 0.21	95.1 ± 1.10 b	89.4 ± 3.29‡	0.99	0.98
0.1 RD	3.9 ± 0.10	2.9 ± 0.31*	1.5 ± 0.22	1.1 ± 0.35	96.1 ± 1.81 b	91.3 ± 4.32	0.99	0.99
SL	4.2 ± 0.60	2.6 ± 0.29*	1.5 ± 0.34	1.2 ± 0.10	93.7 ± 2.56 ab	90.0 ± 4.40	0.99	0.99
DW	3.9 ± 0.92	3.2 ± 0.48	1.6 ± 0.15	1.0 ± 0.06†	97.0 ± 0.71 b	85.7 ± 4.86‡	0.99	0.99
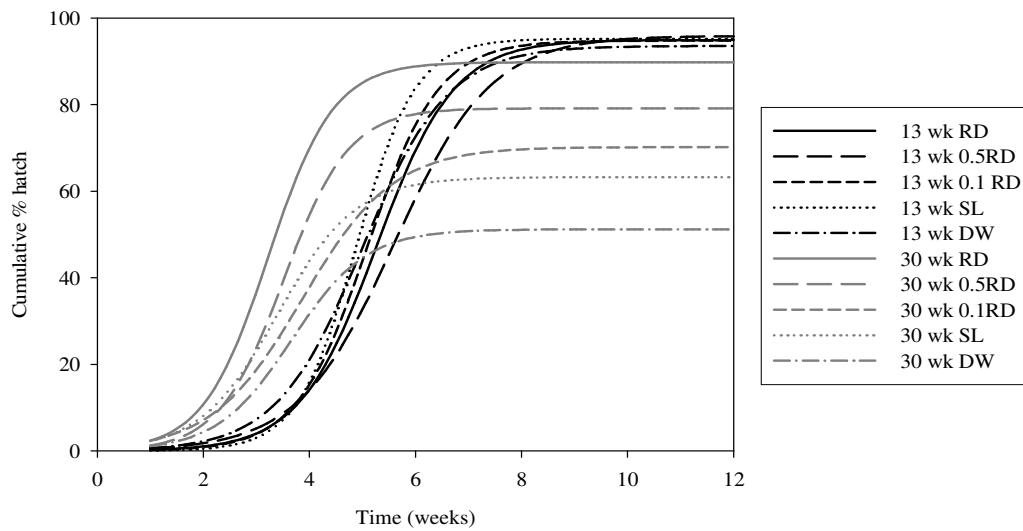
Figure 4.1: Fitted curves showing the cumulative percentage hatch in distilled water (DW), soil leachate (SL), tomato root diffusate (RD) and 50% and 10% concentrations of RD from egg masses of *Meloidogyne chitwoodi* collected from 13 week and 30 week-old tomato plants.
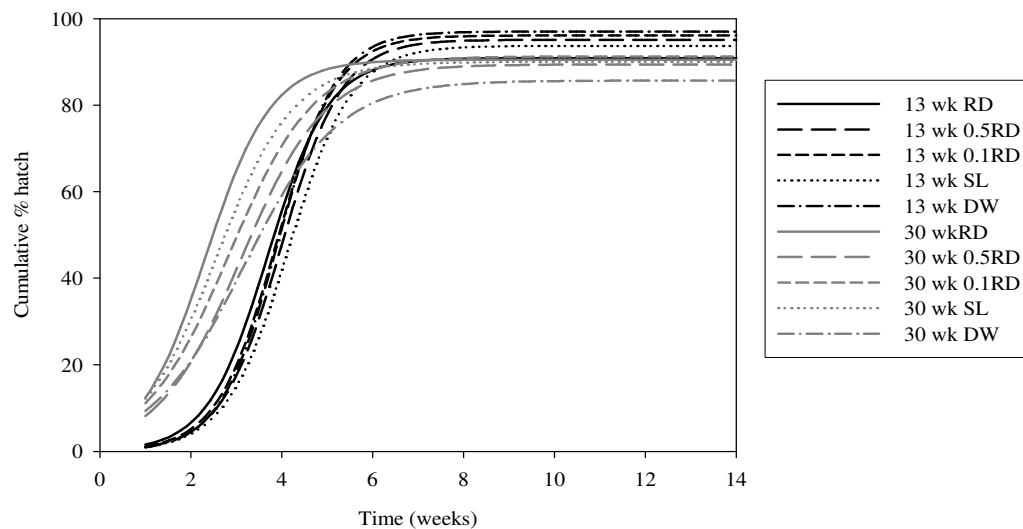
Figure 4.2: Fitted curves showing the cumulative percentage hatch in distilled water (DW), soil leachate (SL), tomato root diffusate (RD) and 50% and 10% concentrations of RD from egg masses of *Meloidogyne fallax* collected from 13 week and 30 week-old tomato plants.

For *M. fallax* in general, no effect of root diffusates occurred (Table 4.2; Fig.4.2). Egg masses collected from 13 wk-old plants gave a significantly lower percentage hatch in RD compared with 0.5RD, 0.1RD and DW. There were no significant differences in parameters m and b.

4.3.2   Effect of host plant age on hatch

In RD, 0.5RD and 0.1RD, J2 of *M. chitwoodi* hatched significantly slower (m) from egg masses collected from 13 wk-old plants compared with those taken from 30 wk-old plants (Table 4.1). Also, in SL and DW parameter m was greater for eggs collected after 13wk, but was not significant. The rate of hatching (b) was significantly higher in 0.5RD for 30wk. Parameter c was significantly lower for 30wk in 0.5RD, 0.1RD, SL and DW. In DW the percentage hatch declined to 51.2%.

J2 of *M. fallax* hatched significantly slower from egg masses from 13 wk-old plants in RD, 0.1RD and SL compared with those from 30 wk-old plants (Table 4.2). Also, in 0.5RD and DW parameter m was greater for eggs collected after 13wk but, again, was not significant. The rate of hatching was significantly lower for J2 from 30 wk-old plants in DW. The final hatching percentage was lower for J2 from egg masses collected on 30 wk-old plants but the difference was only significant in 0.5RD and DW. The lowest percentage hatch was recorded in DW but it still reached 85.7%

4.3.3   Comparison between *Meloidogyne chitwoodi* and *M. fallax*

The average number of eggs per egg mass of *M. chitwoodi* was significantly lower for egg masses from 30-week-old plants compared to egg masses from 13-week-old plants (Fig. 4.3). The average number of eggs per egg mass of *M. fallax* was greater on senescing plants, but the difference between the numbers on young and old plants was not significant.

For egg masses taken from 13-week-old plants, *M. fallax* achieved 50% hatching more rapidly than *M. chitwoodi* but the difference between species was only significant for treatments with 0.5RD and 0.1RD. The rate of hatching was greater for *M. fallax* in 0.5RD. The final hatching percentage was the same for both, except in RD where the final percentage hatch of *M. chitwoodi* (94.9%) was slightly greater than that for *M. fallax* (90.9%).
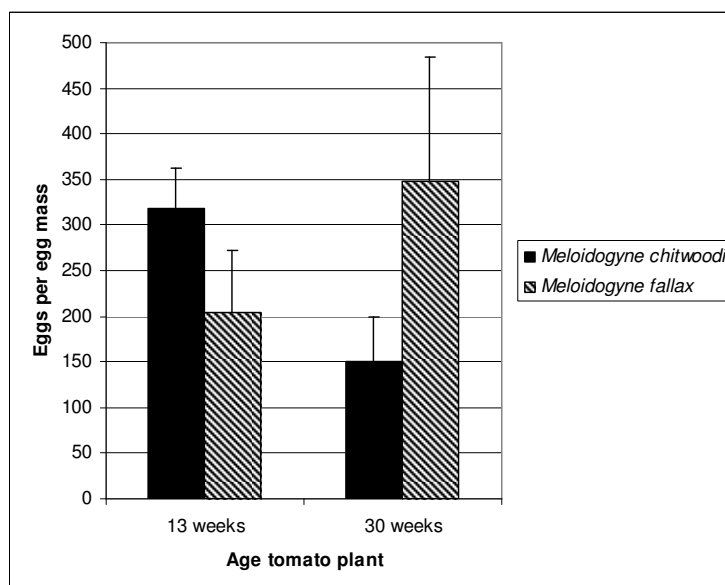
Figure 4.3: The average number of eggs per egg mass of *Meloidogyne chitwoodi* and *M. fallax* collected from 13 week and 30-week old tomato plants. Bars represent the standard deviation.

Egg masses collected on 30 wk-old plants gave a significantly lower final percentage hatch for *M. chitwoodi* in 0.5RD, 0.1RD, SL and DW compared with *M. fallax*. There were no significant differences in parameter b. *Meloidogyne fallax* hatched more rapidly than *M. chitwoodi* but the difference was only significant for treatment with 0.1RD.

4.4 Discussion

Hatching of juveniles of *M. chitwoodi* from egg masses taken from young, actively growing tomato plants was not influenced by the presence of tomato root diffusates. Not only the final percentage hatch, but also the rate of hatching and the time at which 50% hatch was reached were equal in all treatments. This confirms the work of Inserra *et al*. (1983) who found no persistent and stable action of the root leachates of potato, tomato and wheat on the hatching of *M. chitwoodi*. J2 of species of *Meloidogyne* hatch when environmental conditions are favourable and root diffusates are not required for hatching of most species, although they can enhance the rate of hatching (Perry, 1997).

However, there is a change in the hatching physiology of J2 produced on plants at the end of the growing season. The hatch from egg masses collected from senescing tomato plants was significantly less in distilled water than in 0.1RD, 0.5RD and RD. In soil leachates hatch was significantly lower than in 0.5RD and RD. The presence of root

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diffusates increased the hatch of juveniles substantially but had no effect on the rate of hatching or the time at which 50% hatch was reached. It seems that J2 that required RD for hatch needed to be exposed to it for a certain time period before hatching commenced. Hatching continued until 12 weeks after the start of the experiment but the majority of the J2 hatched within the first 6 weeks. Therefore, these J2 are more likely to be in obligate quiescence rather than in diapause, where the J2 would be refractory to RD stimulation.

Temperature plays an important role in the development of *Meloidogyne* (de Guiran & Ritter, 1979; Inserra *et al.*, 1983; Pinkerton *et al.*, 1991; Ploeg & Maris, 1999). Plant growth until senescence and incubation of the egg masses were conducted under controlled optimum temperature regimes. Therefore, the induction of obligate quiescence in J2 of *M. chitwoodi* from senescing tomato plants is directly linked with the condition of the host plant. The production of quiescent J2, which depend on host root diffusate for hatching, ensures survival during short intercrop periods in summer.

By contrast, *M. fallax*, although closely related to *M. chitwoodi*, shows a different hatching pattern. In general, root diffusates did not affect the hatching of J2 from egg masses taken from young or senescing tomato plants. In all cases, hatch of 86% or more was recorded.

Hatching of J2 of *M. chitwoodi* and *M. fallax* from egg masses collected on old senescing tomato plants started more quickly than the hatching of J2 from egg masses taken from young plants. The females inside the root pieces from the young tomato plants were well nourished. Although the collected root pieces contained clearly visible egg masses, it is possible that some females had only begun to produce eggs at the start of the hatching assay. Other females were still actively producing eggs. It is likely that this continued during the beginning of the experiment, thus giving a mixture of eggs in different stages of embryogenic development, which may have influenced the hatching curves for 13 weeks. The eggs collected from senescing plants were embedded in a brown egg mass, the females inside the deteriorated root pieces were dead and no new eggs were produced. Ishibashi (1969) considered that old or poorly nourished females of *M. incognita* produce brown egg masses containing dormant eggs, which are resistant to environment stresses and nematicides and which hatch under the stimulus of root diffusates. Young and well nourished females produce white egg masses susceptible to environmental stresses but from which J2 hatch spontaneously. Although *M. chitwoodi* shows the same pattern, even in the presence of RD, a small proportion (6 – 10%) of eggs remained unhatched. In contrast to *M. chitwoodi*, no quiescent J2 were recorded in *M. fallax*, although a small

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proportion of eggs did not hatch (4-14%). These eggs were unembryonated and did not contain developed J2. The percentage of these eggs increased with plant age both for *M. chitwoodi* and *M. fallax*, but the increase was not significant. The arrest of development of eggs occurs in *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* (de Guiran & Villemin, 1980). Evans (1987) suggested the behaviour of delayed embryonation is certainly a survival strategy but cannot be considered as diapause since there is no specific element of timing linked to seasonal stimuli.

Meloidogyne chitwoodi seems to have a strategy for survival in the absence of a host that is based on two cornerstones: quiescent J2, which hatch only in the presence of root diffusates, and delayed development of unembryonated eggs. Gaur *et al.* (2000) have reported the production of three kinds of unhatched J2 of *M. triticoryzae*: *i*) those that hatch freely in water, *ii*) those that require stimulus from host root diffusates, and *iii*) those that do not hatch even in the presence of host root diffusates. The proportion of unhatched J2 of type 3 increased in the final generation; the presence of unembryonated eggs was not reported. In *H. sacchari* (Ibrahim *et al.*, 1993), *H. cajani* (Gaur *et al.*, 1992) and *H. sorghi* (Gaur *et al.*, 1995) hatch from cysts produced on senescing plants was significantly less than hatch from cyst of the earlier generations. Under the given conditions for plant growth, the life cycle of *M. chitwoodi* and *M. fallax* from J2 until egg production was 3 to 6 weeks. Therefore, it is likely that 2 to 4 generations developed and the egg masses from 30 week old plants consist of different generations. The formation of different generations could have had an effect on the presence of quiescent J2 and further research is required on this aspect.

The survival strategy of *M. fallax* seems to be based on delayed development of embryonating eggs and, presumably, the ability of hatched J2 to survive in the soil. The number of eggs per egg mass for *M. fallax* collected on senescing plants was significantly greater than the number of eggs of *M. chitwoodi*. Together with the fact that 90% of the eggs from senescing plants hatched in soil leachate this might indicate that a greater number of infective J2 of *M. fallax* remains in the soil compared with *M. chitwoodi*. If these J2 are able to survive the absence of a host plant and other adverse conditions for a longer time period (winter) they can immediately penetrate host roots when they appear, which gives them an advantage over quiescent J2. However, survival as hatched J2 requires energy, and it will be interesting to determine whether the energy reserves after a winter period are sufficient for invasion. Robinson *et al.* (1987) showed that if *Globodera* spp. utilised 50% of their energy reserves, there was insufficient left to enable successful

invasion. *Meloidogyne chitwoodi* and *M. fallax* both have a wide and similar host range (Santo *et al.*, 1980; O'Bannon *et al.*, 1982; Ferris *et al.*, 1993; Brinkman *et al.*, 1996) and can be present as a mixed population in the same field, although this does not occur often. Further research is required to examine possible competition between these two species and the role of their survival strategies.

The present work has shown interesting differences between the hatching responses of *M. chitwoodi* and *M. fallax*, which could be linked to different survival strategies. Detection of *M. chitwoodi* and *M. fallax* through soil sampling and subsequent incubation has to take into account delayed hatch, the presence of quiescent J2 and unembryonated eggs. Knowledge about the condition of the host plant can help to optimise the incubation conditions and to avoid an underestimation of the nematode population.

Chapter 4

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Chapter 5

Vertical distribution of Meloidogyne chitwoodi under field crops[*]

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5.1 Introduction

Meloidogyne chitwoodi has multiple generations during a crop-growing season and can build up high population levels. However, the population decreases markedly during winter and under fallow (Pinkerton *et al*., 1991; Noling & Becker, 1994). In Belgium, farmers need to prove, through soil sampling, that their fields are free of *M. chitwoodi* before a contract for growing carrots or black salsify is given to them. In general, fields are sampled between December and March to a standard depth of 25 cm. Crops grown on fields declared free of *M. chitwoodi*, based on these sampling practices, were sometimes severely damaged. The presence of nematode populations below detection levels, and extensive sampling and nematode extraction errors can be a reason for non-detection. However, it is also possible that damage was caused by nematodes originating from soil layers deeper than 25 cm. Nematodes can migrate towards the roots of a host plant and deep rooting crops can reach soil layers with a higher density of nematodes. Johnson and McKeen (1973) found that a population of *M. incognita* situated at a depth of 120-125 cm was able to induce galls on tomato roots that were present in the top 15 cm of a sandy loam glasshouse soil. Pinkerton *et al*. (1987) found that *M. chitwoodi* migrated 30 cm upward and the recovered J2 were able to penetrate tomato plants. The authors did not observe a difference in migration in the presence or absence of a suitable host. In a vineyard in California, juveniles of *Meloidogyne* spp. were detected in relatively high numbers 120 cm below the surface and still occurred at 330cm depth (Ferris & McKenry, 1974). Although, Mojtahedi *et al*. (1991) showed that only a small fraction of the *M. chitwoodi* population in a potato field migrated upward and remained infective, this migration was sufficient to cause significant damage to potato tubers in the field.

In this chapter the importance of the vertical distribution of *M. chitwoodi* for their detection by soil sampling in rotations with field grown vegetables is examined. The population density of *M. chitwoodi* in different soil layers was monitored on two fields naturally infected with *M. chitwoodi* during two successive years. The host status of summer barley, carrot, fodder beet, bean and marigold, and the distribution of *M. chitwoodi* at different depths in these field-grown crops are discussed in order to improve sampling schemes.

5.2 Materials and methods

5.2.1 Presampling

Based on sampling results provided by the Flemish Diagnostic Centre for Plants, Merelbeke, Belgium, two fields (sandy soil, organic matter: 2.3-4%, pH: 5.2) naturally infected with *M. chitwoodi* were selected. *Meloidogyne chitwoodi* was the only species of root-knot nematodes present in the fields. Other plant-parasitic nematodes present included *Pratylenchus crenatus*, *P. penetrans*, *Tylenchorhynchus* spp. and *Rotylenchus* spp. In each of the fields the experiments were established in the area with the highest *M. chitwoodi* infection as established by preliminary soil sampling.

5.2.2 Crops and field characteristics

On both fields, maize (*Zea mays*) and summer wheat (*Triticum aestivum*) were cultivated in 2002 and 2003, respectively. The succession of crops used for experimentation on both fields is shown in Table 5.1. Preliminary tests under glasshouse conditions showed that carrot (*Daucus carota* cv. ABK) was a good host, bean (*Phaseolus vulgaris* cv. Polder) a poor host and marigold (*Tagetes patula* cv. Single gold) a non-host for *M. chitwoodi*. Fodder beet (*Beta vulgaris*) and barley (*Hordeum vulgare*) are considered as moderate to good hosts (O'Bannon *et al*., 1982; Ferris *et al*., 1993).

Table 5.1: Succession on the fields selected for data collection.

		Host	Sowing date	Harvest date
Field 1				
	2004	Summer barley, *Hordeum vulgare* cv. Prestige	8th April	12th August
	2005	Carrot, *Daucus carota* cv. ABK	28th April	13th September
Field 2				
	2004	Fodder beet, *Beta vulgaris* (unknown cultivar)	12th April	24th November
	2005	Bean, *Phaseolus vulgaris* cv. Polder	26th May	3rd August
		Marigold, *Tagetes patula* cv. Single gold	3rd August	9th November (mulched + incorporated)

The fields were ploughed in the first week of April 2004 and the second week of April 2005 and April 2006. In between crops the fields were manually kept weed free (black fallow). Fertilization, pesticide applications and irrigation were according to farmers' usual practice.

Data on the mean monthly air temperature were obtained from the Royal Dutch Meteorological Institute (KNMI). The temperature was recorded in a thermometer shelter located at the weather station of Eindhoven, 35 and 36 km from the experimental fields. No data on soil temperatures or rainfall were collected.

5.2.3 Experimental design and sampling

In each field an experimental plot of 20 m × 2 m was set out. From 14 April 2004 to 27 April 2005 stratified soil samples were taken every 3 weeks, afterwards samples were taken every 4 weeks. At each sampling date, 15 soil cores (2.5 cm diameter, 70 cm depth) were taken along the plant rows. From 14 April 2004 to 27 April 2005 five replicates were taken, afterwards 4. Each core was divided into seven segments of 10 cm (Fig. 5.1). For each replicate, the 15 segments from corresponding depths were pooled.
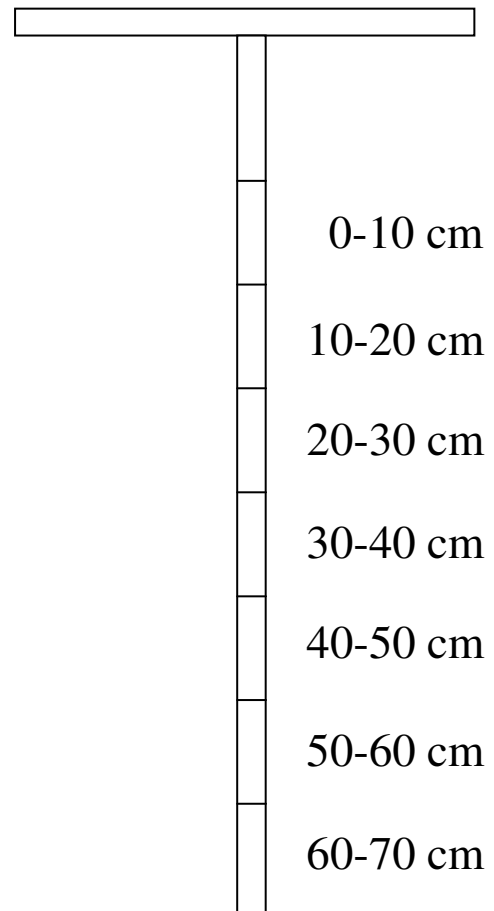
Figure 5.1: Sampling core and division into segments.

5.2.4   Sample processing

Nematodes were extracted from both soil and mineral fraction with the zonal centrifuge (see 3.3.2). After extraction the nematodes were collected in a glass beaker. Nematodes from mineral and organic fraction were pooled after extraction.

5.2.5   Nematode counts

Juveniles and adults of *M. chitwoodi* were counted. Eggs were not counted because of the presence of other plant-parasitic nematodes. Eggs could not be distinguished from each other based on their morphology. Nematode eggs look the same irrespective of the size off the adult (Perry, 2002) and therefore the abundance of *M. chitwoodi* eggs could not be measured; only the motile hatched stages and adults were counted. The results were expressed as nematodes per 100 g soil.

5.2.6   Host plant status

To determine the host plant status, the nematode counts of the sampling dates closest to the sowing and harvest of the different crops represented the initial (Pi) and the final (Pf) population, respectively.

5.2.7   Statistical analysis

The total number of *M. chitwoodi* juveniles and adults per soil layer were expressed as percentages of the total number found in the soil profile between 0 and 70 cm. A multifactor analysis of variance (ANOVA) was carried out to determine the effect of sampling date, soil layer and the interaction between both on the total percentage nematode values.

The mean cumulative percentages of *M. chitwoodi* were fitted to the logistic model $Y = 100/ (1 + \exp(-b \times (d-m)))$, where Y is the cumulative percentage *M. chitwoodi* at soil depth d, 100 is the total cumulative percentage over all soil layers (100%), b is the slope of the curve, and m is the soil depth where 50% cumulative percentage of nematodes is obtained. For the statistical analyses Statistica 7 was used.

5.3 Results

5.3.1   Plant growth and development

There were no visible above–ground symptoms in either of the crops. Root galls were detected on the roots of barley, fodder beet and carrot. At harvest time, 15% of the carrots

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showed severe quality damage on the tap-root, but the size or weight of the roots were not different from uninfected carrot roots (data not shown). Fresh roots were found up to 70 cm depth under summer barley, fodder beet and carrot and up to 60 cm depth under bean and marigold. From the middle of the fallow periods till the following crop, roots were limited to the first 50 cm.

5.3.2   Host plant status

Figure 5.2 shows the mean total numbers of *M. chitwoodi* (juveniles + adults) found in the examined soil profile from 0 to 70 cm depth and the mean air temperature at Eindhoven (source: KNMI, The Netherlands) from spring 2004 until spring 2006. In field 1 the population of *M. chitwoodi* decreased during spring 2004. Under summer barley the population increased but did not reach the same numbers as in early spring. Under black fallow during the following autumn and winter the population decreased, but in spring 2005 a small peak appeared. Soon after this peak the numbers of *M. chitwoodi* continued to decrease, even after the sowing date of carrot. Under carrot the population increased near the end of the growing season and reached the highest numbers at the time of harvest. During the autumn of 2005 the level of nematodes stayed high but decreased in the following winter. In spring 2006 a peak appeared.

In field 2 the initial population increased remarkably under fodder beet at the end of the summer 2004 but started to decrease before the harvest of the crop. In spring 2005 a peak appeared after ploughing. Afterwards the population decreased and this continued under bean and marigold.

To determine the host plant status of the crops, the population densities before sowing (Pi) and immediately after harvest (Pf) were compared for the different soil layers separately (Fig. 5.3 & 5.4). For summer barley Pf was lower than Pi in all soil layers. No nematodes were found after the harvest in the soil layers 50-60 and 60-70 cm. For carrot, Pf was considerably higher than Pi for the soil layers between 0 and 50 cm. In the layers from 50 to 70 cm no nematodes were found. In field 2 fodder beet gave a substantial increment of the population of *M. chitwoodi* in all soil layers except the layer 0-10 cm. The Pf for bean and marigold was lower than Pi in all soil layers except for the layers 50-60 and 60-70 cm in marigold.

A

B
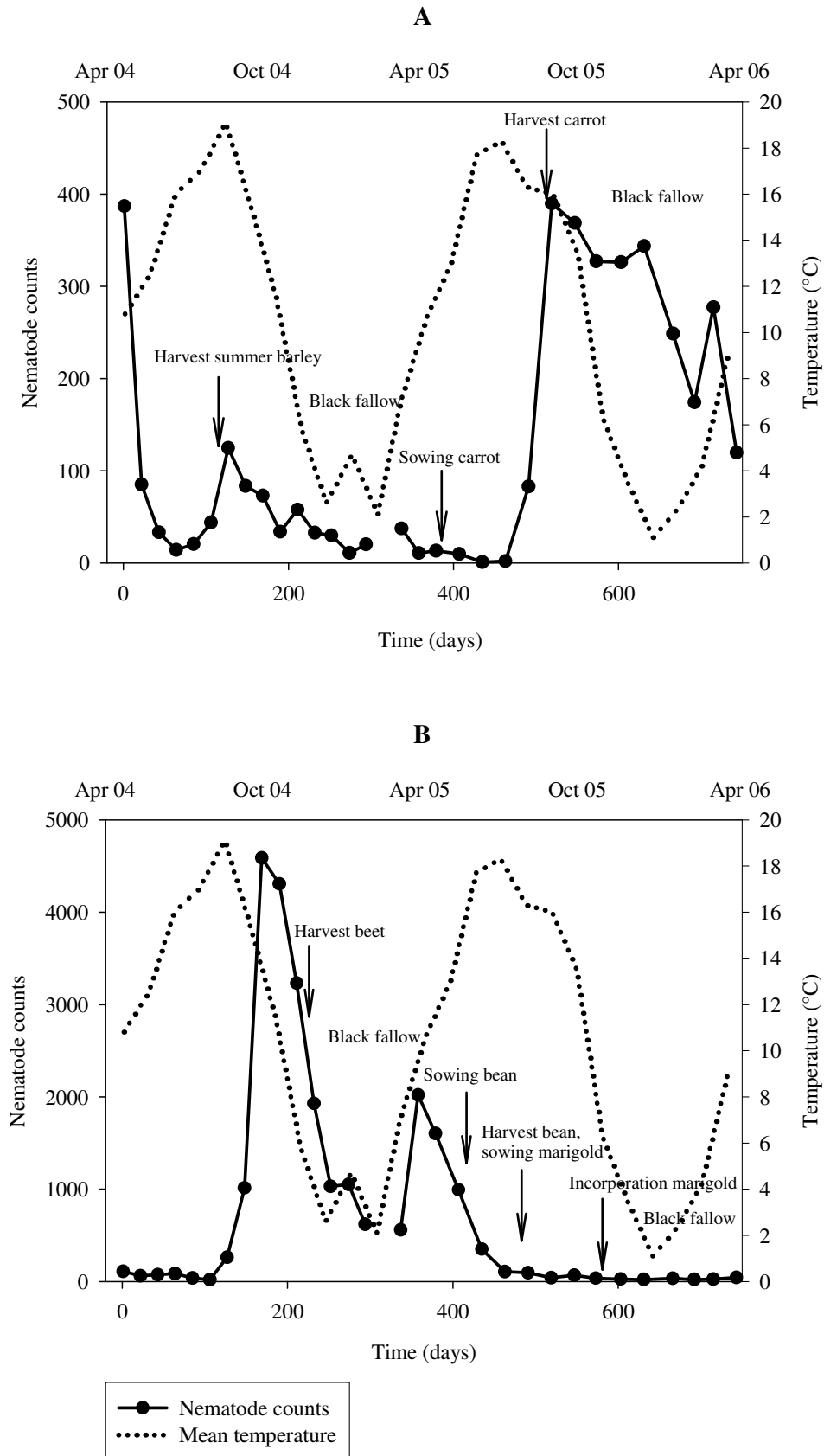
Figure 5.2: Mean total numbers of *Meloidogyne chitwoodi* (adults + juveniles) in the soil profile from 0 to 70 cm depth on field 1 (A) and field 2 (B) and the mean monthly air temperature. Day 1, the first sampling date, is 14 April 2004.

Summer barley (2004)

Carrot (2005)
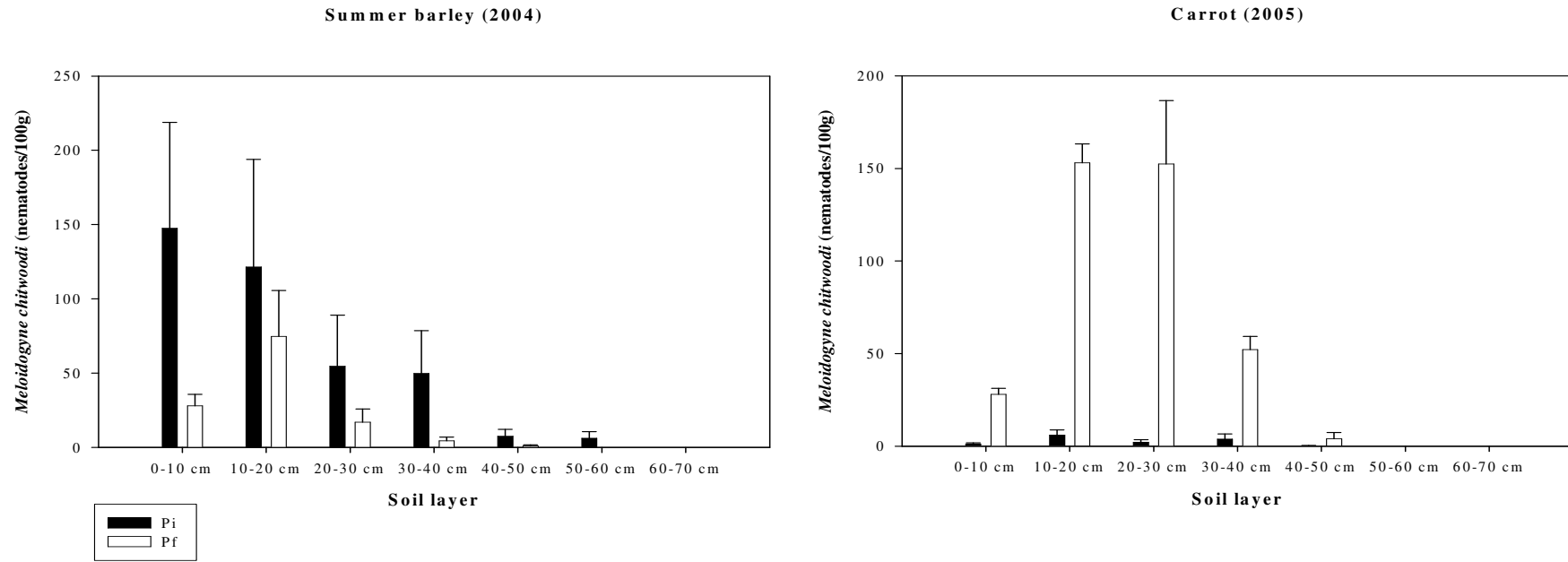
Figure 5.3: The initial (Pi) and final (Pf) population per soil layer (Means ± Standard Error) of *Meloidogyne chitwoodi* for summer barley and carrot (different scales) on field 1.
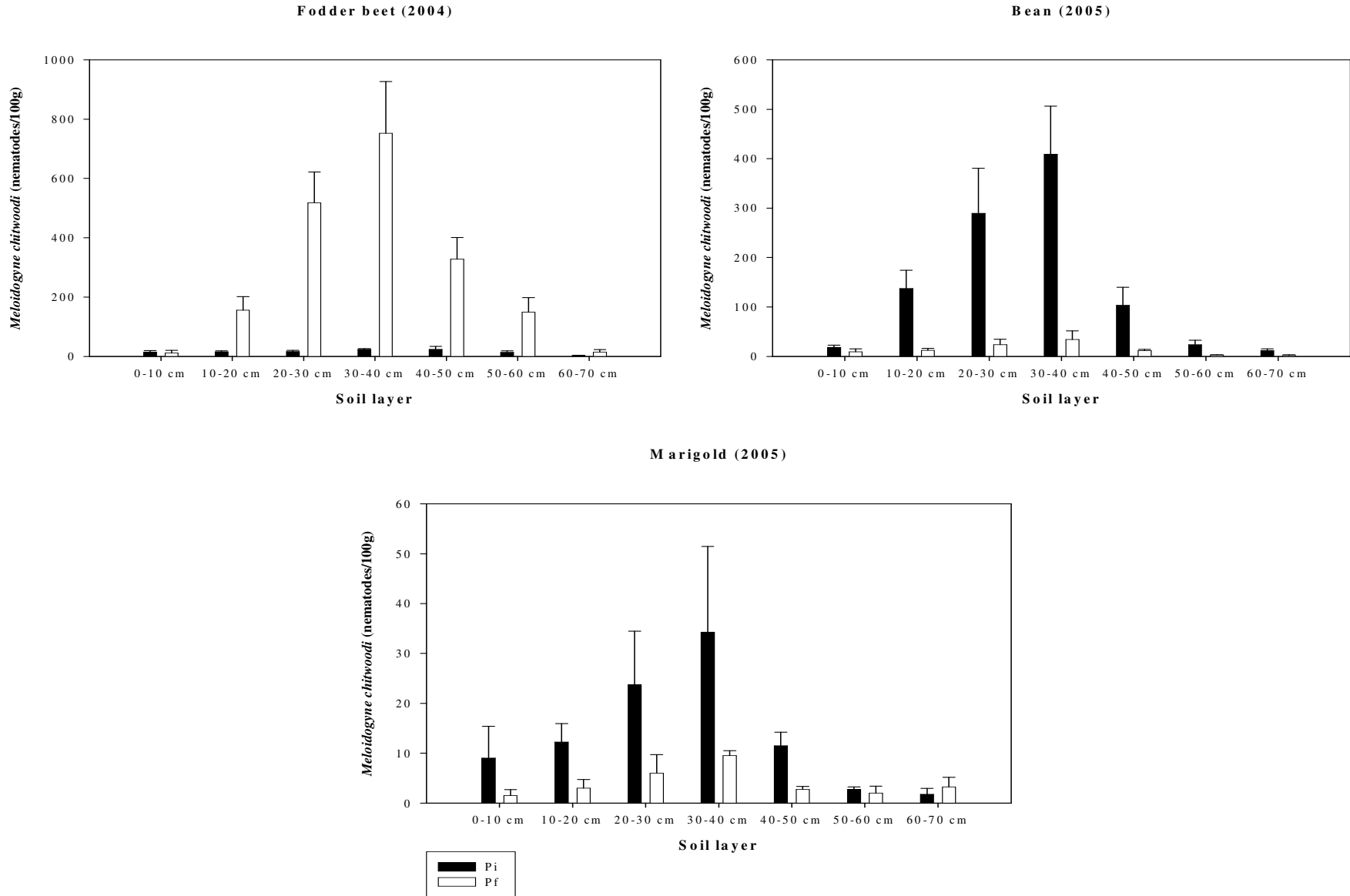
Figure 5.4: The initial (Pi) and final (Pf) population per soil layer (Means ± Standard Error) of *Meloidogyne chitwoodi* for fodder beet, bean and marigold (different scales) on field 2.

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5.3.3   Distribution of *Meloidogyne chitwoodi* over different soil layers

The densities of *M. chitwoodi* for each soil layer separately for field 1 and 2 are shown in figure 5.5. In field 1 nematode densities were highest in the soil layer 10-20 cm under summer barley. After carrot the highest densities were present in the layer 20-30 cm. Ploughing in spring 2006 brought more nematodes in the layer 0-10 cm. In field 2 the highest numbers of *M. chitwoodi* were found in the layer 30-40 cm under fodder beet. After ploughing in spring 2005, layer 20-30 cm contained the highest number of nematodes. On both fields, in general, the nematode densities followed a similar pattern for all soil layers, although there seemed to be a delay in the deeper soil layers. The multifactor ANOVA performed on the nematode counts per soil layer, expressed as percentages, did not show any significant effect of the sampling date (Table 5.2) for both fields. Based on the calculated P values of the F test, both soil layer and the interaction between soil layer and sampling date were significant sources of variation in nematode densities in both fields. However, the variance explained by the interaction was very small compared with the variance explained by the soil layer. Therefore, I concluded that increases and decreases in population densities of *M. chitwoodi* took place simultaneously in the different soil layers and that the percentages could be averaged over time for further analysis.

The mean cumulative percentages of nematodes at increasing soil depth were described by a logistic model. Parameter b indicates the steepness of the slope and parameter m represents the required soil depth to detect 50% of the nematodes. Higher values of m indicate a deeper distribution of nematodes. Figure 5.6 shows the models for the different crops and intercrop (fallow) periods and the overall model for fields 1 and 2. The models for the crops and intercrop periods were calculated based on the sampling data collected between the sowing dates and the dates of harvest of the crops. The values of parameters b and m are shown in table 5.3. In field 1 soil sampling to a depth of 19.6 cm was required to detect 50% of the *M. chitwoodi* population. Parameter m was highest for the fallow period after carrot and lowest for the fallow period after summer barley. The slope of the curve (b) was highest for carrot and lowest for summer barley. In field 2, soil sampling to a depth of 33.5 cm was required to detect 50% of the *M. chitwoodi* population. Parameter m was highest in the fallow period after marigold and lowest in the fallow period after fodder beet. Parameter b was highest in the fallow period following beet.

Table 5.2: Significance of main and interaction effects of variables for the vertical distribution of *Meloidogyne chitwoodi* in two infected fields under two different successions.

Source of variation	*F* test	*P* value
Field 1		
Sampling date	0.138	1.000
Soil layer	164.064	0.000
Sampling date × soil layer	2.282	0.000
Field 2		
Sampling date	0.000	1.000
Soil layer	147.915	0.000
Sampling date × soil layer	2.099	0.000

Table 5.3: Parameters of the logistic model $Y = 100/(1 + \exp(-b \times (d-m)))$ fitted to the cumulative percentages of nematodes present in the soil layers, where Y is the cumulative *% Meloidogyne chitwoodi* at soil depth d. Means ± the standard error of the soil depth where 50% cumulative percentage of nematodes is obtained (m) and of the slope of the curve (b).

	m	b
Field 1		
Summer barley	19.7 ± 0.70	0.131 ± 0.0116
Fallow 1	18.2 ± 0.35	0.184 ± 0.0111
Carrot	18.5 ± 0.61	0.260 ± 0.0417
Fallow 2	22.4 ± 0.26	0.189 ± 0.0084
Total model	19.6 ± 0.23	0.171 ± 0.0065
Field 2		
Fodder beet	34.7 ± 0.43	0.127 ± 0.0061
Fallow 1	29.5 ± 0.35	0.158 ± 0.0079
Bean	32.2 ± 0.51	0.139 ± 0.0089
Marigold	34.9 ± 0.66	0.122 ± 0.0087
Fallow 2	37.1 ± 0.52	0.120 ± 0.0066
Total model	33.5 ± 0.24	0.130 ± 0.0036
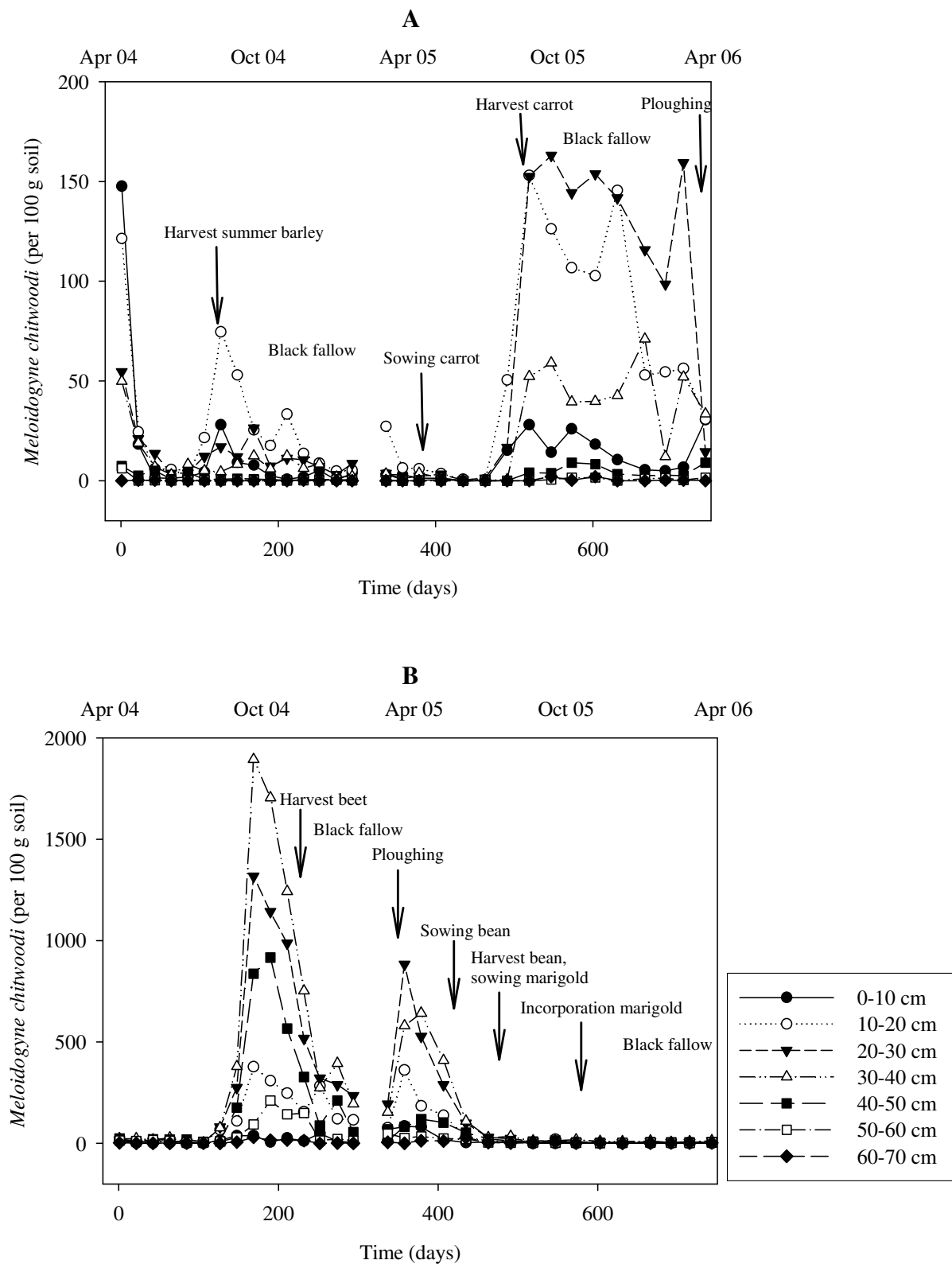
Figure 5.5: The mean numbers of *Meloidogyne chitwoodi* (adults + juveniles) per 100 g soil in different soil layers on field 1 (A) and field 2 (B). Day 1, the first sampling date, is 14 April 2004.

A

$$Y=100/e^{-0.172(d-19.6)} \quad R^2 = 0.81$$

Legend (A):
- — ·· — Summer barley
- — — — Fallow 1
- — — Carrot
- ········· Fallow 2
- —— Field 1

X-axis: **Soil depth (cm)**
Y-axis: **Cumulative % *Meloidogyne chitwoodi***

B

$$Y = 100/e^{-0.130(d-33.5)} \quad R^2 = 0.88$$

Legend (B):
- — · — Fodder beet
- — — — Fallow 1
- — — Bean
- ········· Marigold
- — ·· — Fallow 2
- —— Field 2

X-axis: **Soil depth (cm)**
Y-axis: **Cumulative % *Meloidogyne chitwoodi***
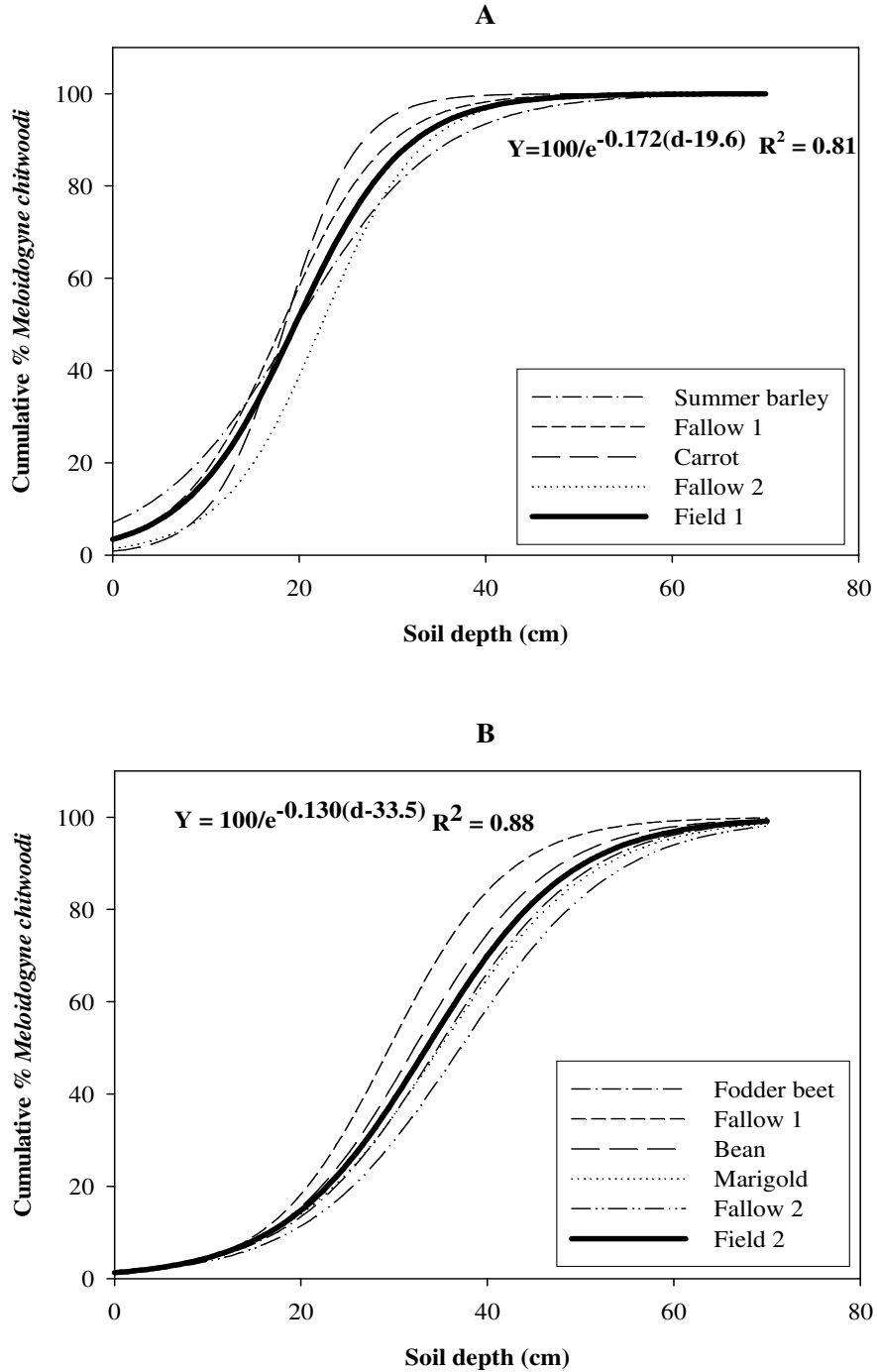
Figure 5.6: The logistic models fitted to the cumulative percentages of *Meloidogyne chitwoodi* present in the soil layers on field 1 (A) and field 2 (B). Explanation of model parameters in described in text (R^2 with P < 0.001).

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5.4 Discussion

   Preventative soil sampling to detect infestations with *M. chitwoodi* is very important to avoid quality damage to field grown vegetables. The results presented in this chapter show that the relative distribution of *M. chitwoodi* over the different soil layers examined in two fields was consistent during two successive years. The different successions with good, moderate and poor hosts did not influence this distribution significantly. For each field a logistic model could be fitted to the cumulative percentages. Based on these models the required depth to detect a given percentage of the *M. chitwoodi* population could be calculated. In field 1 the distribution was shallower than in field 2. In the two years before the starting date of the in-depth soil sampling the same crops (maize and summer wheat) were grown on both fields. Therefore, the difference in vertical distribution in the two fields is most likely due to reasons other than crop rotation. Mojtahedi *et al*. (1991) found that the ability of *M. chitwoodi* to migrate and cause damage appeared to depend on soil texture. Soil with a higher silt and/or clay content may hinder the motility of root-knot nematodes (Prot & Van Gundy, 1981). Although both fields were categorized as sandy soils, field 2 contained a greater sand fraction, the moisture level in each soil layer was higher compared to field 1, and the water table increased faster (data not shown). These features might enable *M. chitwoodi* to survive winter through migration and acclimation to greater depths. Our data showed no evidence of *M. chitwoodi* moving to deeper layers during colder spells.

   Although increases and decreases in population densities of *M. chitwoodi* took place simultaneously in the different soil layers, we cannot conclude that no migration took place. Therefore, more knowledge is required about the number of eggs in the soil profile, their survival and the rate of hatch of juveniles. It is possible that migration of J2 was masked by continuous hatching. Starr and Jeger (1985) found that eggs are as important as J2 in winter survival of *M. incognita* and *M. arenaria*. They reported an increase in numbers of J2 during the early winter months while eggs and the total nematode population declined. Viable eggs were detected up to March. Pinkerton *et al*. (1991) reported that second-stage juvenile densities of *M. chitwoodi* after potato, declined through winter and increased slightly as soil temperatures increased in the spring. The decline continued soon after this peak. A similar pattern was recorded in my fields. The peaks in the population densities recorded in spring on both fields can be explained by hatching of juveniles from eggs. After these peaks the densities continued to decline. *Meloidogyne*

species are obligate parasites and in the absence of a host their numbers decline. For summer barley and carrot the highest nematode numbers (adults + juveniles) were found immediately after harvest. However, in fodder beet, the number of *M. chitwoodi* decreased before the harvest date. The field period of summer barley and carrot is much shorter than that of fodder beet. The low temperature requirements of *M. chitwoodi* for reproduction (O'Bannon & Santo, 1984; Griffin, 1985) enable more generations to be formed on crops with long field periods. The later generations can be formed on crops that are starting to senesce. In chapter 4 I demonstrated that egg masses of *M. chitwoodi* collected from senescing tomato plants contained a percentage of unhatched J2 that required root diffusate to cause hatch and 6-10% remained unhatched. This pattern could be a reason for the decline of the population in fodder beet before the harvest date and the slow decline after carrot. Nematode eggs present in the soil and changes in the hatching of juveniles from eggs during the growing season and the successive fallow could have influenced the results. However, high aggregation of eggs in the soil (Been & Schomaker, 2006) can result in great variation.

The activity and presence of plant-parasitic nematodes are correlated to the distribution of the root system (Ingham *et al.*, 1985; Verschoor *et al.*, 2001). The final population densities (Pf) I found were greatest in the soil layers corresponding to the highest root densities for summer barley, fodder beet and carrot but this did not change the relative vertical distribution. The cultivars of bean and marigold used in this work were poor or non hosts. In crop rotations with poor or non hosts and fallow in winter, the field period of host plants might be too short to influence the vertical distribution of a population of *M. chitwoodi* that is already established in the field. In monocultures it is more likely that the vertical distribution is closely related to the root system of the host plant. Rodríguez-Kábana and Robertson (1987) suggest a direct relation between juvenile numbers of *M. arenaria* and the root density of peanut on light soil texture in a field that had been continuously cropped with peanut as a winter crop for 10 years.

Based on the results from the present work, I would advise farmers to take soil samples immediately after harvest, especially after crops with a long field period. Samples taken soon after harvest gave the highest detection chances for *M. chitwoodi*. This was also found by Been *et al.* (2002) in a potato field. As the time after harvest increases, the numbers of juveniles in the soil decrease and detection becomes more difficult. Incubation could increase the chances for detection of small population densities but is time

consuming (see chapter 4) and raises the costs. Detection based on the presence of juveniles and adults enhances the speed of diagnosis. Therefore, I suggest adapting the depth of the cores taken to the vertical distribution of the population. The results suggest that this distribution is persistent in crop rotations and depending on field characteristics. However, a longer observation of *M. chitwoodi* populations under different crop rotations and in fields with different soil characteristics is required to develop a better sampling strategy for the detection of this quarantine pest.

Chapter 6

*Quality damage on carrots (***Daucus carota***) caused by* Meloidogyne chitwoodi[*]

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6.1 Introduction

Carrot (*Daucus carota*) is an important vegetable, both for the fresh market and the processing industry, and is cultivated worldwide. The production area in the EU covers 125,689 ha (FAO, 2003). In Belgium, approximately 2,500 ha of carrots are grown annually for industrial processing (Anonymous, 2007). A major production area is located in the sandy soils of the provinces of Antwerp and Limburg where the root-knot nematode *Meloidogyne chitwoodi* is widespread (Waeyenberge & Moens, 2001). Root-knot nematodes *M. chitwoodi*, *M. hapla* and *M. fallax* can cause considerable losses by deforming the carrot taproot. *Meloidogyne hapla* induces galling, forking, hairiness and stubby roots (Vrain, 1982; Widmer *et al*., 1999). Slinger and Bird (1977) reported that only 57% of carrots grown in the presence of *M. hapla* were suitable for fresh market use. They observed detrimental effects as early as 4 days after germination of the carrot seeds. Vrain (1982) found no correlation between initial *M. hapla* densities and the weight of mature taproots in growth chamber conditions. However, in a field plot the initial nematode density was negatively correlated with the weight of the taproots. Less information is available for *M. chitwoodi*. Cultivars of carrot were classified nonhosts, moderate hosts or good hosts depending on the race of *M. chitwoodi* (O'Bannon *et al*., 1982; Mojtahedi *et al*., 1987; Santo *et al*., 1988). Santo *et al*. (1988) reported that in pots plant weights of *M. chitwoodi*-infected carrots were significantly less than uninoculated controls. However, this was not the case in field-grown carrots where detrimental effects on quality were also rarely observed.

Uniformity and quality of the carrot taproot is of major importance for the food canning industry rather than maximum yield. Not only forked or stubby roots, but also taproots with heavy galling cannot be processed. To avoid losses, the food canning industry demands soil sampling to detect root-knot nematode infestations. However, due to shortage of land for carrot cultivation, fields with low densities of *M. chitwoodi* are retained for the culture of carrots. In some cases infested fields are fumigated.

The objectives of the studies presented in this chapter were to evaluate the host suitability of different carrot cultivars for *M. chitwoodi* and to determine the influence of low nematode densities on the infection and the quality damage caused by this nematode. The importance of the period that carrots are kept in the field before harvest was also examined.

6.2 Materials and methods

6.2.1   Screening

To determine the potential of 19 different cultivars of carrot as a host for *M. chitwoodi*, 40 plants per cultivar were individually screened as described in chapter 3 (see 3.4).

6.2.2   Damage experiments

The infection of carrots with *M. chitwoodi* and the damage caused by this nematode were examined in two pot experiments and one field experiment. For the pot experiments, carrots were sown in 4-l pots (Optipot 17 RX) filled with sterilized sandy soil. The soil weight was determined for each individual pot. In each pot five carrots were sown in a circle. Plants were watered when required. Every 3 weeks a liquid fertilizer (NPK 7-4-6, 5 ml/l, Bayer) was supplied. At harvest, both the carrots and the soil were gently removed from the pots leaving the root system intact. The soil was gently washed away and the infection of each individual carrot was visually determined. This was done by examining both galling and the presence of egg masses on the root system. Each carrot plant was given a score: 0 or 1 representing no infection or visible nematode infection, respectively. The infected carrots were further divided in two groups: infected (galling and/or presence of egg masses on the lateral roots) with no visible damage and infected with damage to the taproot. Damaged roots were defined as unprocessable for the canning industry. The percentage of plants with infection and plants with damage were calculated per pot.

The nematodes used in these experiments were taken from the stock culture (see 3.2).

6.2.2.1 Effect of inoculation time

Carrots, cv. Amfine were sown at the end of April. The pots were kept in a plastic tunnel whose sides were kept open from June onwards to reduce the ambient temperature. The pots were inoculated 1, 2, 4 or 6 weeks after emergence of the plants, with inoculation densities of 0, 1, 2, 6, 12, 24 or 48 freshly hatched (< 24 h) *M. chitwoodi* J2/ 100 g soil. The inoculation of nematodes was done by pouring the nematode solution onto the soil surface with an excess of water; the water did not percolate through the drain holes of the pots. There were eight replicates for each treatment (time of inoculation × inoculation density) and the pots were fully randomized. The carrots were harvested 105 days after sowing.

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6.2.2.2 Effect of harvest date

Sterilized sandy soil was infected with different quantities of freshly hatched (< 24 h) J2 of *M. chitwoodi* and mixed thoroughly to obtain nematode densities of 2, 10 or 25 J2/ 100 g soil. Uninfected soil was used for control. Carrots cv. ABK were sown; the pots were fully randomized and kept in a growth chamber. Temperature, light regime and humidity were adapted to the average values during the carrot growing season (Table 6.1). The carrots were harvested 100, 120 or 140 days after sowing. Each treatment (nematode density × time of harvest) was replicated eight times.

Table 6.1: Mean daylight, temperature and humidity during the growing season of carrots in Belgium. Data obtained from the Royal Meteorological Institute (KMI), based on long term means at the weather station of Ukkel.

Time (days)	Daylight (h)	Day temperature (°C)	Night temperature (°C)	Relative humidity (%)
0-30	14	12.7	4.5	76
31-60	15	17.2	8.2	75
61-90	16	19.8	11.0	77
91-120	15	21.8	12.7	78
121-150	14	21.9	12.6	78

6.2.3 Field experiment

In a field (sandy soil, organic matter: 2.3-4%, pH: 5.2), naturally infested with *M. chitwoodi*, 40 m^2 carrots cv. ABK were sown in rows according to farmers' usual practice (22 kg seeds/ha, 3 rows at 7.5 cm distance and 15 cm between groups of 3 rows). The initial (Pi) and final (Pf) population density of *M. chitwoodi* were determined by soil sampling (60 cores, 25 cm depth, and 1.75 cm diam.) before and after the crop. Nematodes were extracted from both the organic (after maceration) and mineral soil fraction of these samples with an automated zonal centrifugal machine as described in chapter 3 (see 3.3.2). At harvest (139 days after sowing), five samples of 250 carrots were taken randomly. Each carrot was visually scored for damage as described above.

6.2.4 Statistical analysis

The percentage of infected carrots and damaged carrots were calculated and cos(x) transformed to fulfil the requirements for ANOVA analysis. The effect of inoculation

density, time of inoculation and time of harvest were determined with a multifactor ANOVA. An exponential regression model $Y = m(1 - \exp(-bX))$, where Y is the percentage infection or damage found with nematode density X, m is the maximum infection or damage found with nematode density 25 J2/ 100 g soil and b is the slope of the curve, was fitted to relate inoculation density with percentage infection, and damage of carrot cv. ABK with each of the three harvest times. Statistica 7 was used for the statistical analyses.

6.3 Results

6.3.1 Screening

Figure 1 shows the mean number of egg masses of *M. chitwoodi* per tested plant that were found on each of the carrot cultivars. Egg masses were found on both the young taproots and secondary roots and were produced on all cultivars. However, there were no egg masses on more than 80% of the plants of cvs Berlanda, Bolero, Chantenay, Nantucket and Parmex (data not shown). The mean number of egg masses per infected plant of these cultivars was less than three. The greatest mean number of egg masses were found on cvs ABK, Douceur, Maxi and Merida. Egg masses were formed on each plant of these cultivars.
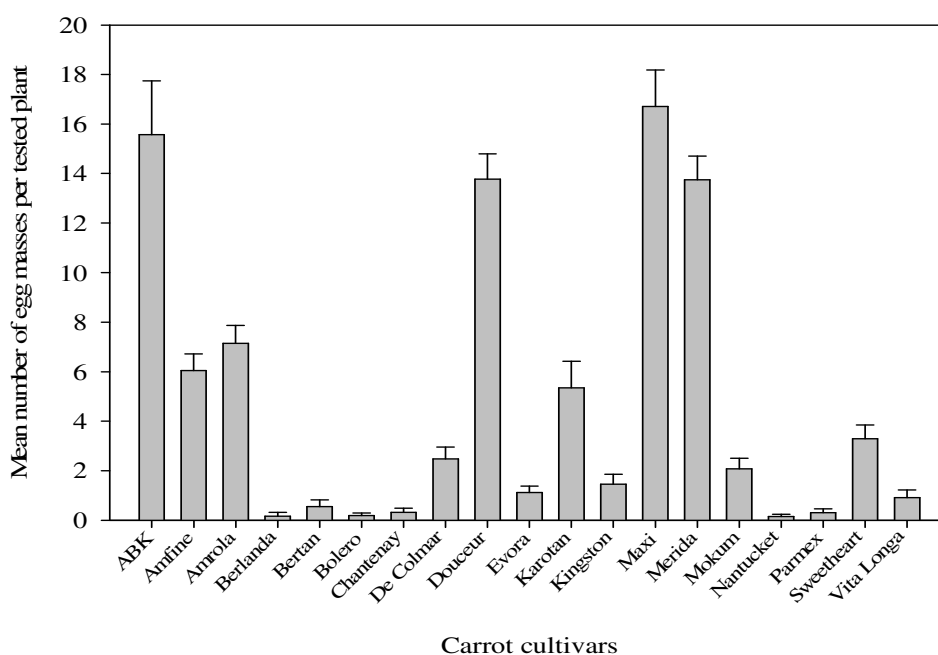
Figure 6.1: The mean number of egg masses produced on 19 different cultivars of carrot 8 weeks after inoculation with 200 second-stage juveniles of *Meloidogyne chitwoodi*. The vertical bars represent the standard error of the mean.

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6.3.2 Damage experiments

Visibly infected carrots showed galls on the taproot and on the secondary roots. Egg masses were clearly visible. Unprocessable, damaged carrots were characterized by a rough surface of the taproot caused by severe galling near the lenticels of the root (Fig 6.2). In non-inoculated control plants no damage was evident.

Figure 6.2: Quality damage on the taproot of carrot caused by *Meloidogyne chitwoodi.*

6.3.2.1 Effect of inoculation time

For the infection of carrots, the time of inoculation, the inoculation density and the interaction between both factors were significant sources of variation (Table 6.2). However, the variance explained by the interaction effect was small compared with the variance explained by the time of inoculation and the inoculation density. Infection of the carrots was positively correlated with both inoculation density ($r = 0.29$, $P < 0.01$) and the time of inoculation ($r = 0.26$, $P < 0.01$). For damage, time of inoculation and inoculation density were significant effects (Table 6.2). Damage was positively correlated with inoculation density ($r = 0.23$, $P < 0.01$) and time of inoculation ($r = 0.14$, $P < 0.01$).

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Table 6.2: Significance of main and interaction effects of variables for the infection of carrots cv. Amfine and the damage caused by *Meloidogyne chitwoodi*.

Source of variation	*F* test	*P* value
Infection		
Time of inoculation	41.582	0.000
Inoculation density	28.314	0.000
Time of inoculation × Inoculation density	1.868	0.015
Damage		
Time of inoculation	12.501	0.000
Inoculation density	11.428	0.000
Time of inoculation × Inoculation density	1.279	0.194

The percentage of carrots (cv. Amfine) that were infected (including those that were damaged) and the percentage of carrots that were damaged 105 days after sowing are shown in table 6.3. Inoculation with nematode densities ≤ 24 J2 1 week after the carrot plants emerged per 100 g soil resulted in maximum 20% infection. No damage was visible with nematode densities of 1 or 2 J2 per 100 g soil. Inoculation with 48 J2/ 100 g soil gave 40% infection and 12.5% damage. Inoculation 2 weeks after the emergence of the carrot plants resulted in 25% to 45% infection. Five percent damage was observed with the lowest inoculation density (1 J2/ 100 g soil). However, no damage was recorded with inoculations of 6 J2/ 100 g soil. The maximum damage was 12.5% after inoculation with 48 J2/ 100 g soil. Inoculation with 48 J2/ 100 g soil 4 weeks after emergence resulted in 65% of infected carrots and 15% damage. Inoculation after 6 weeks gave infection percentages between 52.5% and 82.5%. A minimum of 10% of the carrots were damaged, with a maximum of 40% when inoculated with 48 J2/ 100 g soil.

Table 6.3: The mean percentage of *Meloidogyne chitwoodi* infected carrots (± standard error of the mean) and the percentage of unprocessable, damaged taproots (± standard error of the mean) 105 days after sowing. The carrots (cv. Amfine) were inoculated at different times after emergence with different nematode densities. Infection and damage were visually determined.

Inoculation density (J2/ 100 g soil)	Inoculation time (weeks after emergence)							
	1		2		4		6	
	Infection (%)	Damage (%)	Infection (%)	Damage (%)	Infection (%)	Damage (%)	Infection (%)	Damage (%)
1	10.0 ± 4.80	0.0 ± 0.00	27.5 ± 7.15	5.0 ± 3.49	10.0 ± 4.80	0.0 ± 0.00	60.0 ± 7.84	12.5 ± 5.30
2	12.5 ± 5.30	0.0 ± 0.00	25.0 ± 6.93	2.5 ± 2.50	17.5 ± 6.08	2.5 ± 2.50	52.5 ± 8.00	10.0 ± 4.80
6	12.5 ± 5.30	2.5 ± 2.50	32.5 ± 7.50	0.0 ± 0.00	27.5 ± 7.15	2.5 ± 2.50	55.0 ± 7.97	10.0 ± 4.80
12	20.0 ± 6.41	10.0 ± 4.80	35.0 ± 7.64	5.0 ± 3.49	30.0 ± 7.34	5.0 ± 3.49	57.5 ± 7.92	12.5 ± 5.30
24	17.5 ± 6.08	7.5 ± 4.21	35.0 ± 7.64	10.0 ± 4.80	30.0 ± 7.34	7.5 ± 4.21	65.0 ± 7.64	22.5 ± 6.69
48	40.0 ± 7.84	12.5 ± 5.30	45.0 ± 7.97	12.5 ± 5.30	65.0 ± 7.64	15.0 ± 5.72	82.5 ± 6.08	40.0 ± 7.84

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6.3.2.2 Effect of harvest date

For the infection of carrots, only the inoculation density showed a significant source of variation (Table 6.4). The infection was positively correlated with the inoculation density (r = 0.70, P < 0.01). For damage, both inoculation density and time of harvest had significant effects (Table 6.4). Damage was positively correlated with the inoculation density (r = 0.41, P < 0.01) and the time of harvest (r = 0.28, P < 0.01).

Table 6.4: Significance of main and interaction effects of variables for the infection of carrots cv. ABK and the damage caused by *Meloidogyne chitwoodi*.

Source of variation	*F* test	*P* value
Infection		
Inoculation density	63.057	0.000
Time of harvest	1.019	0.364
Inoculation density × Time of harvest	0.566	0.757
Damage		
Inoculation density	*12.417*	*0.000*
Time of harvest	*7.313*	*0.001*
Inoculation density × Time of harvest	*1.479*	*0.192*

Table 6.5: The mean percentage of *Meloidogyne chitwoodi* infected carrots (± standard error of the mean) and the percentage of unprocessable, damaged taproots (± standard error of the mean) inoculated with different nematode densities and harvested at different times after sowing. Infection and damage were visually determined.

Inoculation density (J2/ 100 g soil)	Time of harvest (days after sowing)					
	100		120		140	
	Infection (%)	Damage (%)	Infection (%)	Damage (%)	Infection (%)	Damage (%)
2	30.0 ± 15.28	0.0 ± 0.00	30.0 ± 15.28	10.0 ± 10.00	50.0 ± 16.67	20.0 ± 13.33
10	70.0 ± 15.28	20.0 ± 13.33	90.0 ± 10.00	50.0 ± 16.67	90.0 ± 10.00	60.0 ± 16.33
25	100.0 ± 0.00	10.0 ± 10.00	100.0 ± 0.00	60.0 ± 16.33	100.0 ± 0.00	70.0 ± 15.28

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The percentage of carrots (cv. ABK) that were infected (including those that were damaged) and the percentage of damaged carrots are shown in table 6.5. In all treatments carrots were found infected with *M. chitwoodi*. All carrots sown in soil inoculated with 25 J2/ 100 g were infected, irrespective of the time of harvest. The percentage of damaged taproots caused by this nematode density increased from 10% when harvested 100 days after sowing to 70% after 140 days. Inoculation density of 10 J2/ 100 g soil resulted in 70% (100 days) and 90 % (120 and 140 days) infection and 20, 50 and 60% damaged taproots 100, 120 and 140 days after sowing, respectively. At the lowest nematode density (2 J2/ 100 g soil) 30% (100 and 120 days) and 50% (140 days) infection occurred. No damaged taproots were found when the carrots were harvested after 100 days. After 120 and 140 days, 10 and 20% of the carrots were damaged, respectively.

An exponential model was fitted to the infection percentages (Fig. 6.3A) and to the damage percentages for each time of harvest separately (Fig. 6.3B). The model for the infection described 62% of the variance with 99% being the maximum infection and 0.21 the slope of the curve. The models for the damage on the carrot taproot described 99% of the variance for the harvest 120 and 140 days after sowing and 81% for the harvest 100 days after sowing. The maximum percentages of damaged taproots were 14, 64 and 71% for harvest 100, 120 and 140 days after sowing, respectively. The slope of the curve was greatest for harvest after 100 days (0.23) meaning that the maximum of the reported damage was reached quickest for this time of harvest compared with harvest after 120 days (0.13) and 140 days (0.18).

There was no effect of nematode infection on the length, width and weight of the carrot taproot (data not shown).

6.3.3 Field experiment

The initial *M. chitwoodi* density before sowing was 3 J2/ 100 g soil. The final population density just after harvesting the carrots was 111 J2/ 100 g. Nematode infection was visible in 25.5% ± 3.42% (SE) of the yielded carrots and 11.5% ± 4.12% (SE) of the carrot taproots were damaged by *M. chitwoodi*.

A

Y = 99(1 - e^{-0.21X})   R^2 = 0.62

Nematode infection (%)

Inoculation density (J2/ 100 g soil)

B

Y = 71(1 - e^{-0.18X})   R^2 = 0.99

Y = 64(1 - e^{-0.13X})   R^2 = 0.99

Y = 14(1 - e^{-0.23X})   R^2 = 0.81

Damage (%)

Inoculation density (J2/ 100 g soil)

●   100 days
○   120 days
▼   140 days
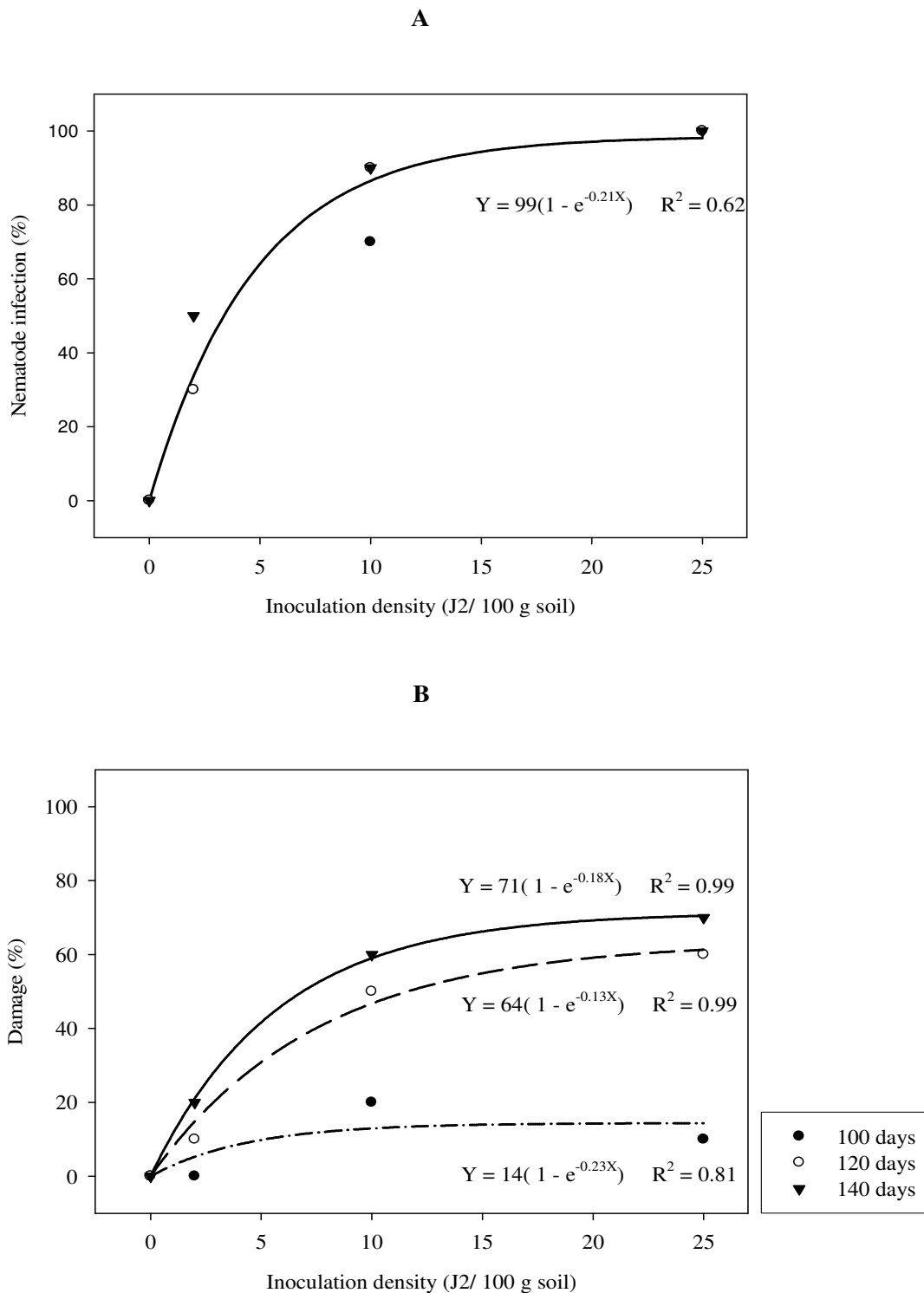
Figure 6.3: The exponential model fitted to the percentage infection (A) and the percentage damaged taproots (B) caused by different population densities of *Meloidogyne chitwoodi* on carrot cv. ABK harvested 100, 120 and 140 days after sowing.

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6.4 Discussion

Based on the screening results, I found great variation in host status of different cultivars of carrot for *M. chitwoodi*. Consequently, the choice of cultivar can play an important role in avoiding substantial *M. chitwoodi* population build ups. The majority of the tested plants of cvs Berlanda, Bolero, Chantenay, Nantucket and Parmex were found without egg masses. This indicates that these cultivars have potential for resistance. However, diversity within a nematode species can result in different responses from crops. van der Beek *et al.* (1998) reported heterogeneity of pathogenicity within *M. chitwoodi* populations but no or little intraspecific specialization. By contrast, Santo *et al.* (1988) showed that diverse populations of *M. chitwoodi* reproduced differently on carrots and an extremely high variability amongst populations was reported on pepper (Berthou *et al.*, 2003). In the United States three races of *M. chitwoodi* are distinguished based on differential host tests (Mojtahedi *et al.*, 1988; Mojtahedi & Santo, 1994). *Meloidogyne chitwoodi* race 1 reproduced on carrot cv. Red Cored Chantenay, in contrast, race 2 did not (Mojtahedi *et al.*, 1988). I did not characterize the population of *M. chitwoodi* used in my experiments based on a differential host test. van der Beek et al. (1999) showed that there was no evidence for the existence of race 2 in The Netherlands. Waeyenberge (1999) reported differences in reproduction factors on carrot of *M. chitwoodi* populations collected at different locations in Belgium but all populations were able to reproduce. Obviously, it is important to screen for resistance for *M. chitwoodi* using populations of different origin or mixed populations, collected from infected fields in the main carrot production areas. The use of resistant carrot cultivars can be an important strategy to overcome major problems. Potential resistance was found in several of the tested cultivars and it is suggested to do further screening in field experiments.

The quality of the taproot is of major importance for the processing industry. In contrast with *M. hapla*, which induces forking, hairiness and stubby roots (Vrain, 1982; Widmer *et al.*, 1999), I observed that *M. chitwoodi* caused severe galling near the lenticels resulting in a rough surface of the carrot taproot. This was also reported by Molendijk (2000). Lenticels are spongy regions in the periderm of stems and roots that allow gas exchange with the surrounding environment. Earlier, Santo *et al.* (1988) allocated similar symptoms to heat cancer, which occurs when carrot seedlings are exposed to temperatures above 20°C (Crete, 1977).

The minimum temperature requirements for carrot growth are 5°C with an optimum between 18 and 25°C (Krug, 1997); seedlings can even tolerate a frost of -7°C (Anonymous, 2004). As carrots are well adapted to cool temperatures, advancing the sowing date could be an option to overcome severe infection and damage in fields with low *Meloidogyne* densities. The hypothesis for this is that the carrot taproot would be better developed and, therefore, less vulnerable to nematode infection. Early planting has been suggested as a cultural practice to reduce *M. hapla* damage to carrots (Brzeski & Bujda, 1974). However, compared with *M. hapla*, *M. chitwoodi* has lower temperature requirements for activity and reproduction (Santo & O'Bannon, 1981; Inserra *et al*., 1983; O'Bannon & Santo, 1984; Griffin, 1985). Inserra *et al*. (1983) demonstrated that at 7°C the emergence of *M. chitwoodi* juveniles from eggs was 7 times greater than that of *M. hapla*. Dormant nematode eggs that survived winter will hatch when environmental conditions are favourable (Perry, 1997). Therefore, advancing sowing carrots to February or March might give an immediate food supply for the freshly hatched juveniles.

This study demonstrates that both infection and damage of carrots by *M. chitwoodi* were not only positively correlated with the inoculation density, but also with the developmental stage of carrot. The percentages of both infected and damaged carrots (cv. Amfine) were greatest for the later infection (6 weeks after emergence). Even with low nematode densities I observed more than 50% infection. In contrast, when the time between emergence and inoculation was short (1 week), only the highest inoculation density (48 J2/ 100 g soil) caused more than 20% infection. It is probable that J2s were not able to locate the root tips of the very poorly branched root system during the early developmental stages of carrot. Six weeks after emergence the root system is much better developed although secondary roots are still not abundant and root-hair development is poor (Weaver & Bruner, 1927). Based on these findings, and considering the low temperature requirements of *M. chitwoodi*, I suggest that advancing the sowing date is not advisable. This strategy would not yield the desired results.

Postponing sowing would leave hatched nematodes without a host and lead to a decline of the population. Molendijk and Brommer (1998) reported that this strategy allowed the production of good quality carrots in fields that were heavily infested with *M. fallax*. As possible explanations they suggest a considerable natural decline of the nematode population before sowing and a faster development of the taproot at higher temperatures.

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However, there are major physiological differences between *M. fallax* and *M. chitwoodi* (chapter 4), so field data on *M. fallax* may not be applicable to *M. chitwoodi*.

Ehwaeti *et al.* (2000) demonstrated that damage by *M. incognita* was density dependent and increased with increasing duration of plant growth. This finding is confirmed by my results for *M. chitwoodi*. Carrots harvested 100 days after sowing gave 14% damaged taproots with initial population densities of 25 J2 of *M. chitwoodi* per 100 g soil. Postponing the harvest up to 120 and 140 days caused an increase of damaged taproots to 64% and 71% damage, respectively. Damage to the carrot taproots seems to develop towards the end of the growing period. The percentage of infected carrots was similar irrespective the time of harvest. *Meloidogyne chitwoodi* can have multiple generations during one crop-growing season. It might be possible that second or third generations of *M. chitwoodi* developed inside the carrot taproot and were responsible for the severe galling. Further research is required to confirm this aspect.

Second-stage juveniles of *Meloidogyne* spp. primarily enter roots directly behind the root cap but penetration can occur also at other sites such as points were lateral roots emerge, penetration sites of other juveniles and cut surfaces of roots (Hussey, 1985). Pinkerton *et al.* (1991) observed that J2s of *M. chitwoodi* do not penetrate young potato tubers unless wounds are present or lenticels are fully developed. Charchar (1987) reported that J2 of *M. chitwoodi* were found below or near the lenticels on the potato tuber surface immediately after penetration, indicating that they penetrated through the lenticels. When the carrot taproot becomes mature, lenticels are formed. Most likely second or third generations of *M. chitwoodi* enter the lenticels inducing damage in the later stage of the growing season.

Reducing the period that carrots are in the field by advancing the harvest date might significantly reduce the proportion of quality damage caused by *M. chitwoodi*. However, the optimal time of harvest is also determined by other quality features. The most important are size, shape, uniformity, colour, texture and internal aspects such as taste, texture and nutritional value (Mazza, 1989). Harvesting at the end of the season resulted in a higher yield with less storage losses and a better quality of carrots (Suojala, 2000). Advancing the harvest date is possible when the sowing density is reduced, but decreases the yield by 15 to 20 t ha^{-1} rendering the carrots more expensive (M. Willocx, La Corbeille, Belgium, pers. comm.).

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In my field experiment with a low initial population of *M. chitwoodi* (3 J2/ 100 g soil) only 11.5% damage occurred 139 days after sowing. In the pot experiments 20% damage was observed 140 days after sowing with initial nematode populations of 2 J2/ 100g soil. Under field conditions nematodes are vulnerable to adverse conditions and natural enemies, which explains the lower degree of infection and damage compared to my pot experiments.

Where there is a shortage of fields that are free of *M. chitwoodi*, one might consider growing carrots when nematode densities are low rather than disinfecting fields. The latter is environmentally unfriendly, costly and cannot guarantee a *M. chitwoodi*-free field. Also the taste and texture of carrots could be negatively influenced by soil fumigation and phytotoxicity might occur (Hutchinson *et al*., 1999). It is suggested that the harvest time should be advanced in order to avoid severe quality damage by *M. chitwoodi* if other quality requirements are fulfilled. An exponential model, like those that were fitted to my data, could help to make decisions on the harvesting time to avoid substantial damage caused by *M. chitwoodi*. Therefore, more data should be collected for a wider range of initial nematode densities, different populations of *M. chitwoodi*, different environmental circumstances and more carrot cultivars.

Given the fact that *M. chitwoodi* infection of carrots was high even with low initial nematode densities, I would advise that carrots should not be grown in *M. chitwoodi*-infested fields in order to avoid huge population build ups. If carrots have to be grown because of a shortage of non-infested fields, cultivars that allow only lower *M. chitwoodi* reproduction can be chosen. Quality damage caused by *M. chitwoodi* appears late in the growing season and can be limited by a reduction of the field period.

Chapter 7

*Host suitability of common bean (***Phaseolus vulgaris)** *for* **Meloidogyne chitwoodi**

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7.1 Introduction

The use of resistant cultivars is an important strategy to overcome major problems with plant pathogens. Growing resistant crops offers an environmental friendly alternative for pest management. In nematology plant resistance has increased in importance with the phasing out of soil fumigants. However, resistance is not widely used. Cook and Starr (2006) suggest four possible reasons: some reports of resistance may not have been accurate; the currently available resistance is often linked to undesirable characteristics; the costs of developing resistant cultivars are not justified by the importance of the nematode problem; and naturally occurring genetic resistance tends to be too specific for use in intensive agriculture. Also the durability of resistant cultivars can be limited. Long-term use of nematode resistant cultivars can induce shifts in races within nematode species resulting in different pathotypes (Young, 1992).

Dealing with resistance, tolerance and host suitability requires a clear definition of these terms. Cook and Evans (1987) defined a plant that allows no nematode reproduction as completely resistant and a plant that allows nematodes to multiply freely as non-resistant or susceptible. They describe a tolerant plant as a plant that suffers little injury even when heavily infected with nematodes and an intolerant plant as a plant that suffers much injury. Resistance and tolerance are independent qualities of a host plant. Host suitability is less well defined. The host suitability or host plant status is often divided in different categories from excellent host, good host and moderate host to poor host and non host. This classification can be made based on different features. The two most commonly used methods for *Meloidogyne* spp. are: the nematode reproduction factor and the egg mass index. The reproduction factor is calculated by dividing the final nematode population density after exposure of the plant to nematodes (Pf) by the initial population density (Pi). If Pf/Pi > 1 than the plant is considered a host, when Pf/Pi < 1, the plant is often categorized as non host. However, the latter does not necessarily mean that nematodes were not able to reproduce. A large Pi may result in a small Pf due to strong competition between nematodes (Cook & Evans, 1987). Therefore, monitoring the development of egg masses is a better method to see whether root-knot nematodes were able to reproduce. Moreover, it is easier because no nematode extraction is required and direct observation is possible.

Resistance against *Meloidogyne* spp. has been reported in apricot, common bean, cotton, cowpea, grape, groundnut, lucerne, peach, soybean, sweet potato, tobacco, tomato

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and walnut (Cook & Starr, 2006). Resistance for *M. chitwoodi* in wheat was reported by Kaloshian *et al*. (1989). More recently resistance for *M. chitwoodi* was found in wild potatoes *Solanum bulbocastanun*, *S. hougasii* (Brown *et al*., 1994) and *S. fendleri* (Janssen *et al*., 1996) and in pepper (Berthou *et al*., 2003). Reports from The Netherlands indicate possible resistance in cultivars of common bean (Molendijk, 2000).

In Belgium, common bean, *Phaseolus vulgaris*, is mainly grown for the freezing and the canning industry. In 2006 a total area of 6096 ha yielded 72,482 tonnes (Anonymous, 2007). Part of the production area is located in the provinces of Antwerp and Limburg were *M. chitwoodi* is widespread (Waeyenberge & Moens, 2001).

In this chapter the host suitability of different bean cultivars for *M. chitwoodi* is discussed based on the ability of the nematode to develop inside bean roots and the production of egg masses. The production of egg masses of *M. chitwoodi* on bean cultivars is compared with two other temperate *Meloidogyne* species, *M. fallax* and *M. hapla*. The influence of *M. chitwoodi* infection on the vegetative plant growth was examined in a pot experiment and the reproduction factor of *M. chitwoodi* on two cultivars was determined under field conditions.

7.2 Materials and methods

7.2.1 Screening

The screening for host suitability of ten cultivars of common bean was done as described in chapter 3 (see 3.4). *Meloidogyne chitwoodi* and *M. fallax* J2 were obtained from the stock culture (see 3.2). *Meloidogyne hapla* J2 were collected from tomato plants grown in *M. hapla*-infested soil that was obtained from the Flemish Diagnostic Centre for Plants. The 10 tested bean cultivars were Cantare, Flagrano, Fulvio, Jamaica, Lipsos, Masai, Mercana, Polder, Proton and Verbano.

Cultivar Polder was observed in a second screening for *M. chitwoodi* 10 weeks after inoculation instead of 8 weeks.

7.2.2 Development of *M. chitwoodi* inside bean roots

Based on the screening results, four cultivars of bean, Masai, Mercana, Polder and Verbano, were selected to monitor the penetration and development of *M. chitwoodi* inside the roots.

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The beans were grown in plastic tubes (15 × 20 × 120 mm). The tubes were filled with sterilized soil (see 3.1) and in each tube one seed was sown. Immediately after sowing, each tube was inoculated with 200 freshly hatched J2 (< 24 h) of *M. chitwoodi*. The plants were kept in a temperature-controlled glasshouse (16-22°C) with daily 14 h light period and watered with an atomizer upon requirements. For each cultivar 30 plants were grown. Two, 4 and 6 weeks after inoculation ten plants per cultivar were taken randomly to monitor the presence and the developmental stage of *M. chitwoodi* inside the roots.

7.2.2.1 Staining of nematodes

The roots were stained using the method described by Bird *et al*. (1983) for the detection of nematodes inside plant tissues. Prior to staining, the plants were submerged in water and the soil was gently washed away from the roots. The roots were cut into 1 – 2 cm fragments and put in a 150 ml glass beaker with 50 ml tap water. To obtain a 1.5% NaOCl solution, 20 ml of chlorine bleach (5.25% NaOCl) was added. With occasional agitation the root fragments were kept for 4 min in this solution. Subsequently, the roots were poured on a 250-µm-sieve and rinsed in running water to remove residual NaOCl. The roots were removed from the sieve and transferred to a beaker containing 30 ml tap water. One millilitre of the staining solution (3.5 g acid fuchsin, 250 ml acetic acid and 750 ml distilled water) was added and heated to boiling for 30 s. After cooling to room temperature the roots were poured on a 250-µm-sieve and rinsed in running water to remove excess stain. The root fragments were then heated to boiling in 20-30 ml glycerol and cooled. Root fragments were kept in glycerol in a Petri dish until observation.

7.2.2.2 Counting of stained nematodes

The stained nematodes were counted with the aid of a binocular microscope. The nematodes were divided into 3 groups: second-stage juveniles, swollen juveniles, and female adults (Fig. 7.1).

A
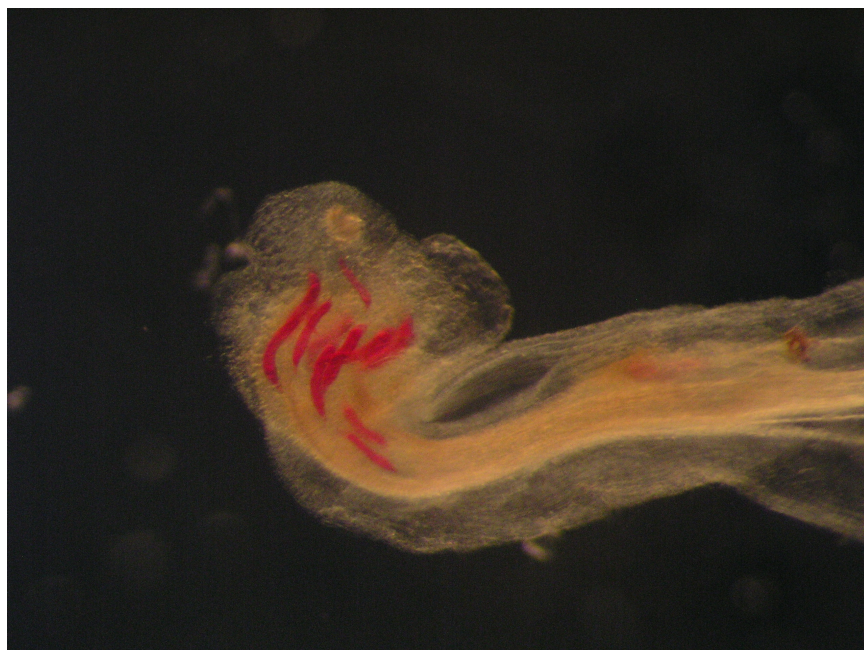
B
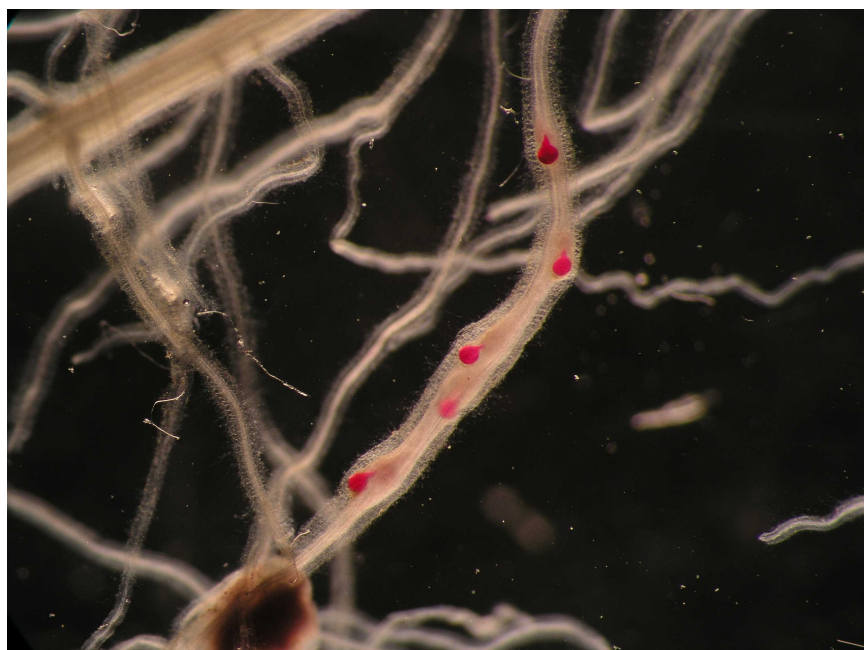
Figure 7.1: Swollen juveniles (A) and females (B) of *Meloidogyne chitwoodi* inside bean roots after staining with fuchsin acid (Bird *et al.*, 1983).

7.2.3 Influence of nematode densities on vegetative growth of bean

Bean cv. Polder was sown in *M. chitwoodi* infested soil with nematode densities of 0, 1, 5, 10, 50, 100, 200 or 1000 J2 per 100 g soil. Therefore 9 cm diam. pots were filled with sterilized soil (see 3.1) and each pot was weighed. The soil was removed from the pot and freshly hatched J2 (< 24 h) were added to obtain the required nematode densities. The infested soil was mixed thoroughly before it was put back in the pot. In each pot one seed was sown immediately afterwards. For each nematode density there were 20 replicates.

The plants were kept in a temperature-controlled glasshouse (20-26°C) with daily 14 h light period. The plants were watered upon requirements and fertilized with a liquid fertilizer (NPK 7-4-6, 5 ml/l, Bayer) every 3 weeks.

After 60 days the plants were weighed and the pods were counted.

7.2.4 Reproduction of *M. chitwoodi* on two bean cultivars under field conditions

In a naturally *M. chitwoodi* infected field, 40 m^2 bean cv. Polder and 20 m^2 bean cv. Verbano were sown on 26th May 2005. The beans were sown in rows 40 cm apart and at 6 – 7 cm between plants. The beans were harvested on 3rd August 2005. The initial (Pi) and final (Pf) population density of *M. chitwoodi* were determined by soil sampling in the middle square meter of the crop just before the sowing date and immediately after harvest, respectively. One soil sample consisted of 60 cores (25 cm depth, 1.75 cm diameter). Nematodes were extracted from both the organic and mineral soil fraction as described in chapter 3 (3.3.2).

7.2.5 Statistical analysis

The screening results were analyzed with factorial ANOVA after log transformation of the data to fulfil the requirements for ANOVA. One-way ANOVA was used after log transformation of the data to analyse the development of *M. chitwoodi* inside bean roots for each time after inoculation separately. The tested bean cultivars were separated with an LSD-test ($P < 0.05$). The influence of the inoculation density of *M. chitwoodi* on the vegetative growth of bean cv. Polder was analyzed with one-way ANOVA. Statistica 7 was used for the statistical analyses.

7.3 Results

7.3.1 Screening

The mean numbers of egg masses of *M. chitwoodi*, *M. fallax* and *M. hapla* per plant 8 weeks after inoculation are shown in figure 7.2. Nematode species, bean cultivar and the interaction between both were significant sources of variation for the production of egg masses (Table 7.1).
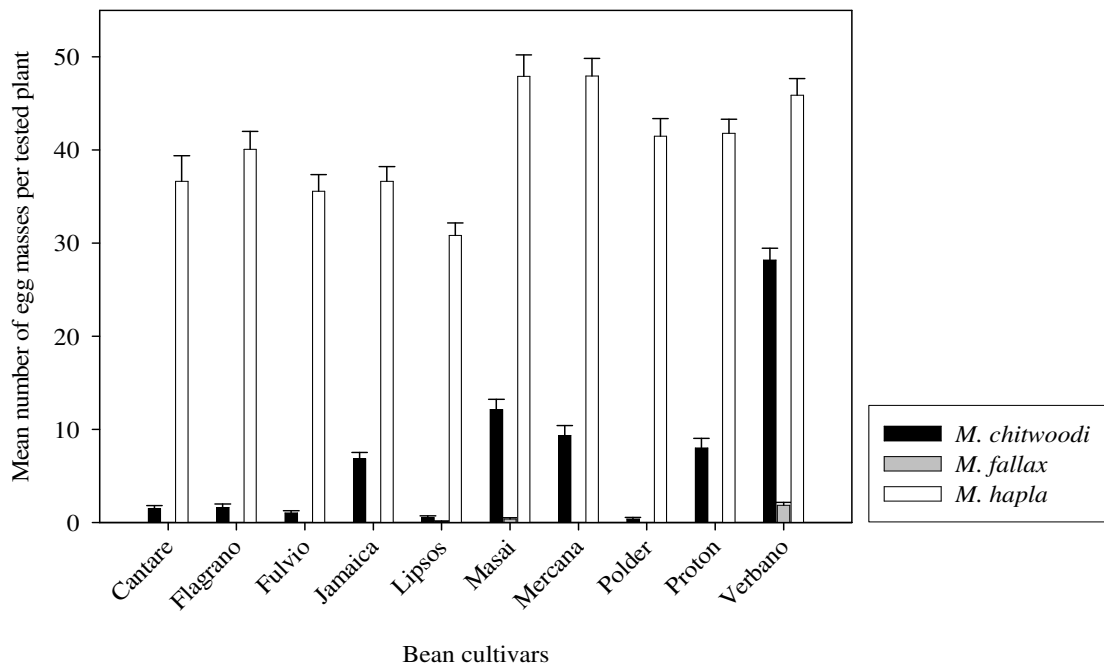
Figure 7.2: The mean number of egg masses per plant of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* of ten cultivars of common bean *Phaseolus vulgaris* 8 weeks after inoculation with 200 J2 per plant. The vertical bars represent the standard error of the mean.

Tabel 7.1: Significance of main and interaction effects of variables for the production of egg masses of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* on different bean cultivars.

Source of variation	*F* test	*P* value
Nematode species	6720.07	0.00
Bean cultivar	131.14	0.00
Nematode species × bean cultivar	62.55	0.00

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Meloidogyne hapla reproduced significantly better than *M. chitwoodi* and *M. fallax* on all of the tested cultivars with a minimum of 30 egg masses per plant (cv. Lipsos). For each cultivar egg masses of *M. hapla* were found on every tested plant.

The different cultivars showed a big variation in the production of egg masses of *M. chitwoodi*. Cultivars Verbano (28.2) and Masai (12.2) give the highest average number per tested plant. In cvs Lipsos and Polder an average of less than 1 egg mass per plant was found. The percentage of the tested plants that contained no egg masses of *M. chitwoodi* are shown in table 7.2.

Table 7.2: Percentage of plants of ten bean cultivars without egg masses of *Meloidogyne chitwoodi* 8 weeks after inoculation with 200 J2 per plant.

Cantare	Flagrano	Fulvio	Jamaica	Lipsos	Masai	Mercana	Polder	Proton	Verbano
47.5 %	45.0 %	55.0 %	5.0 %	72.5 %	0.0 %	5.0 %	82.5 %	7.5 %	0.0 %

The highest percentages of plants with no egg masses were found in cvs Lipsos and Polder. In contrast, on cvs Masai and Verbano egg masses of *M. chitwoodi* were found on all of the tested plants.

The roots of cvs Lipsos and Polder showed clearly visible galls, indicating that *M. chitwoodi* was able to penetrate. Postponing the screening of egg mass production in cv. Polder from 8 to 10 weeks after inoculation resulted in an average of 24.3 ± 1.75 (SE) egg masses per plant and egg masses were found on every plant.

Meloidogyne fallax did not produce egg masses on cvs Cantare, Flagrano, Fulvio, Jamaica, Mercana, Polder and Proton. On cvs Lipsos and Masai 7.5% and 20% of the plants showed egg masses, respectively. On cv. Verbano this increased to 62.5% of the plants.

7.3.2 Development of *M. chitwoodi* inside bean roots

The mean numbers of vermiform, swollen juveniles, and females of *M. chitwoodi* that were found in the roots of bean cvs Masai, Mercana, Polder and Verbano at different times after inoculation are shown in figure 7.3.
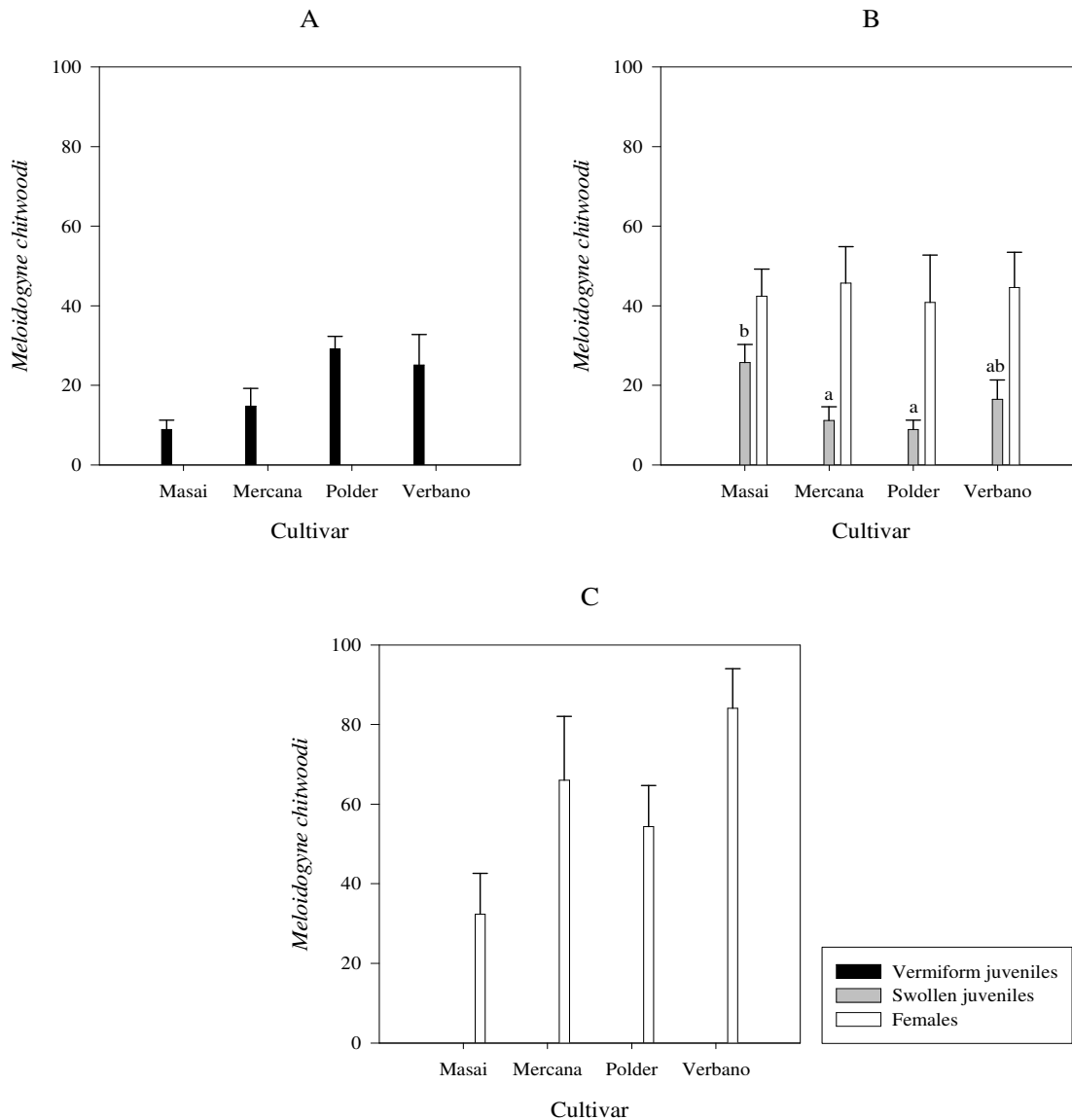
Figure 7.3: Mean number of developmental stages of *Meloidogyne chitwoodi* inside roots of four bean cultivars at different times after inoculation: A, 2 weeks; B, 4 weeks and C, 6 weeks after inoculation. The vertical bars represent the standard error of the mean.

Two weeks after inoculation only vermiform juveniles were found in the four tested cultivars. A greater number of juveniles was detected in cvs Polder and Verbano, but this difference was not significant (F = 2.67, P = 0.06).

After 4 weeks, both swollen juveniles and females were found inside the bean roots. No vermiform juveniles were detected. The number of swollen juveniles was different between cultivars (F = 4.04; P = 0.01). Cultivar Masai contained more swollen juveniles

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than cvs Mercana and Polder. There was no difference in the number of females present in the 4 cultivars.

Six weeks after inoculation only females were found inside the bean roots. Cultivar Verbano contained the highest number of females, but this number was not significantlt different from that in the other cultivars (F = 2.65, P = 0.07).

The total number of *M. chitwoodi* that was found inside the roots of cv. Polder did not change between the observations 2, 4 and 6 weeks after inoculation (F = 0.86; P = 0.43), whereas in cvs Masai (F = 29.52; P = 0.00), Mercana (F = 10.33; P = 0.00) and Verbano (F = 8.18; P = 0.00) the total number of nematodes was significantly higher 4 and 6 weeks after inoculation compared with the number found 2 weeks after inoculation.

7.3.3   Influence of nematode densities on vegetative growth of bean

The mean number of pods produced on bean cv. Polder (Fig. 7.4) was not influenced by the different inoculation densities of *M. chitwoodi* (F = 1.33, P = 0.25).

Equally, there was no significant difference in the aerial growth of bean cv. Polder 60 days after sowing in soil with different *M. chitwoodi* inoculation densities (F = 1.34, P = 0.24) (Fig 7.5).
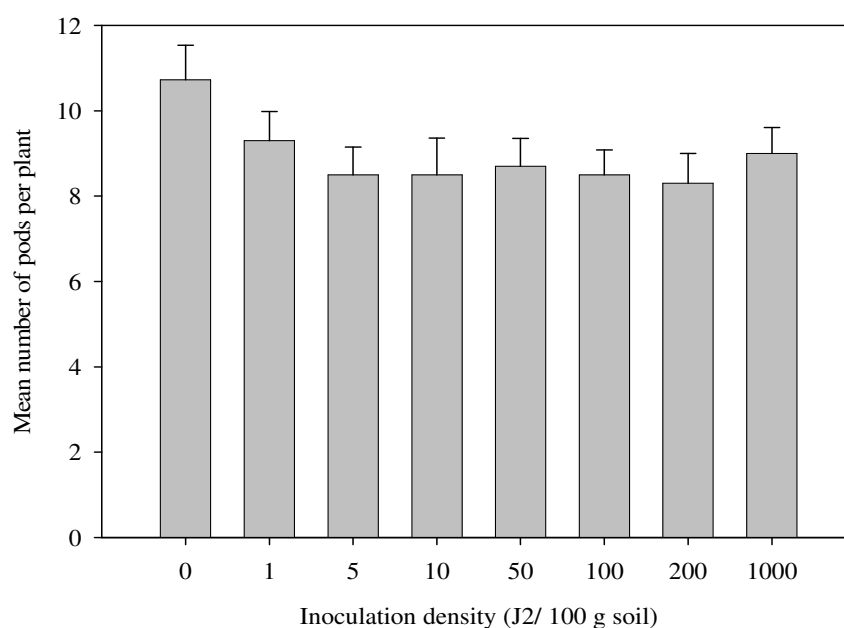
Figure 7.4: Mean number of pods on bean cv. Polder 60 days after sowing in soil infected with increasing densities of *Meloidogyne chitwoodi*. The vertical bars represent the standard error of the mean.
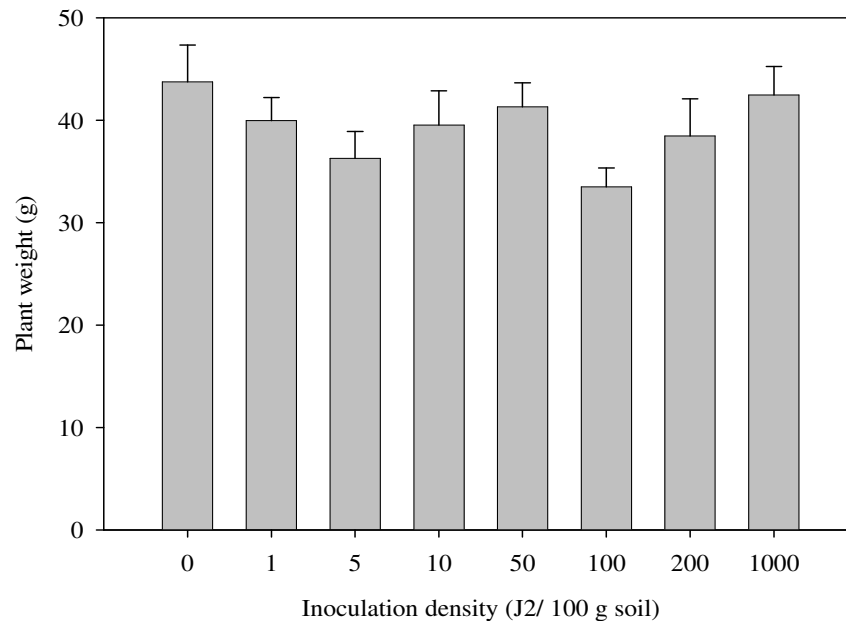
Figure 7.5: Mean weight of the aerial part of bean cv. Polder 60 days after sowing in soil infected with increasing densities of *Meloidogyne chitwoodi*. The vertical bars represent the standard error of the mean.

7.3.4   Reproduction of *M. chitwoodi* on two bean cultivars under field conditions

The total field period was 70 days. The development of both cultivars was normal and no detrimental effects on growth were observed. The nematode densities decreased under cv. Polder (Pf/Pi = 0.78), but increased under cv. Verbano (Pf/Pi = 1.70).

7.4 Discussion

Given the wide host range of root-knot nematodes, decisions on crop rotations for infested fields are difficult and have to be taken cautiously. The screening results from this study showed that the tested cultivars of bean, *Phaseolus vulgaris* L., were poor to good or even excellent hosts for *M. chitwoodi*, non hosts or poor hosts for *M. fallax* and excellent hosts for *M. hapla*. Variations in host suitability for these three *Meloidogyne* species occurred. This confirms earlier studies on host suitability of bean for *M. chitwoodi* and *M. fallax* (Brinkman *et al.* 1996; Molendijk, 2000; den Nijs *et al.*, 2004) and *M. hapla* (Chen & Roberts, 2002). *Meloidogyne hapla* reproduced significantly better on bean than *M. chitwoodi* which was also found by Santo and Ponti (1985). Two cultivars of bean, Lipsos and Polder, showed very few egg masses 8 weeks after inoculation with *M. chitwoodi*.

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More than 70% of the tested plants of these cultivars contained no egg masses, indicating possible resistance, but galls were visible on the roots. The presence of galls on the roots indicated that *M. chitwoodi* was able to penetrate. However, galling does not always imply that nematodes can reproduce and, conversely, reproduction may occur without plant tissue swelling (Cook & Evans, 1987).

Resistance to root-knot nematodes may take effect within the soil environment (preinfectional) or within the root environment (postinfectional), the latter being the most common type (Fassuliotis, 1979). Sydenham *et al.* (1996) found that in bean, resistance to *M. incognita* and *M. arenaria* was expressed by delayed nematode development rather than by differential penetration. I found similar results for the penetration of *M. chitwoodi* but there was no arrested or delayed development of juveniles. Two weeks after inoculation, the presence of J2 inside the roots of cultivars Masai, Mercana and Verbano, where many egg masses were formed 8 weeks after inoculation, was not different from that in cultivar Polder which had allowed the formation of very few egg masses. Similar penetration of resistant and susceptible cultivars by J2 of *Meloidogyne* spp. has also been found in alfalfa (Reynolds *et al.*, 1970; Griffin & Elgring, 1977), maize (Windham & Williams, 1994), cotton (Creech *et al.*, 1995), tobacco (Schneider, 1991) and tomato (Hadisoeganda & Sasser, 1982). After 4 weeks females together with swollen juveniles were found in all cultivars. However, the number of swollen juveniles in cv. Masai was significantly higher than in cvs Mercana and Polder. This could be an indication for delayed development in cv. Masai but it could also be explained by a prolonged penetration of J2. Smaller time intervals than those I used are required to distinguish both possibilities. There was no difference between cultivars for the number of females inside the roots 4 weeks after inoculation. After 6 weeks only females were found inside the roots. This indicates that *M. chitwoodi* was able to develop in all of the four tested cultivars. There was no difference between the cultivars.

In cvs Masai, Mercana and Verbano the total number of *M. chitwoodi* found inside the roots 4 and 6 weeks after inoculation was significantly higher than the number of nematodes found after 2 weeks. In contrast, in cv. Polder the number of nematodes did not change in time, indicating that penetration of juveniles ceased after the initial penetration. Although not significant, the number of juveniles inside the roots two weeks after inoculation was highest in cv. Polder. Unlike many other authors (see above), Minton (1962) and Pedrosa *et al.* (1996) found that initial penetration of roots by J2 was higher in

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resistant cultivars than in susceptible ones but their numbers declined after a few days. Reynolds *et al.* (1970) and Herman *et al.* (1991) reported the emigration of J2 of *M. incognita* from the roots of resistant cultivars of alfalfa and soybean soon after penetration. Resistance related to the absence of certain nutrients may force penetrated juveniles to leave the roots after infection (Huang, 1985).

Based on the initial screening, 8 weeks after inoculation of nematodes, one would wrongly assume the presence of resistance for *M. chitwoodi* in bean cv. Polder. The presence of galls indicated penetration and development of juveniles was confirmed after staining the nematodes inside the roots at different times after inoculation. The penetration and development of *M. chitwoodi* in the four tested cultivars of bean resulted in an equal number of females inside the roots after 6 weeks. Therefore, the significant lower amount of egg masses found on cultivar Polder could not be explained by less penetration or delayed development of nematodes. When cv. Polder was screened 10 weeks after inoculation, egg masses were found on every plant. This indicated that no resistance was present in this cultivar. For plant breeders it is important that the screening method is non destructive. Therefore, staining of nematodes inside plant root tissue is not an option. Postponing the screening date can be a solution if a prolonged growth of the tested plant cultivar is possible.

It seemed that, compared with other cultivars, the formation of egg masses on cv. Polder was delayed. In the field experiment the Pf of *M. chitwoodi* on cvs Polder and Verbano was determined after a field period of 10 weeks. A decrease of the *M. chitwoodi* population was observed on cv. Polder, whereas the population increased on cv. Verbano. This could also be due to a delayed egg mass formation of *M. chitwoodi* on cv. Polder but more research is required on that aspect.

The average field period of beans in Belgium is approximately 65 to 72 days (W. Smeets, Scana-Noliko, Belgium, pers. comm.). Under field conditions the penetration and development of *M. chitwoodi* is most likely slower than under controlled glasshouse conditions. Therefore, bean cv. Polder could be an option for crop rotations in *M. chitwoodi* infected fields. The results presented here, showed no negative effect of *M. chitwoodi* densities up to 1000 J2/ 100 g soil on the vegetative growth of bean cv. Polder. However, climatic conditions with higher temperatures can enhance the development of *M. chitwoodi* (Griffin, 1985). Therefore, I suggest that cv. Polder should be grown as a main crop rather than as an after-crop later in the season. The succession of bean cv. Polder and

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marigold *Tagetes patula* cv. Single Gold reduced the population of *M. chitwoodi* significantly (see chapter 5). When beans are harvested, stubbles and roots remain in the field. This could allow *M. chitwoodi* to survive and continue its development. To avoid this, I suggest that the roots should be destroyed with a rotary cultivator immediately after harvest. In this respect bean cv. Polder can act as a trap crop; it allows *M. chitwoodi* to penetrate and develop and can be destroyed before the nematode is able to reproduce.

Seven of the tested bean cultivars showed no egg mass formation of *M. fallax* 8 weeks after inoculation. In contrast to *M. chitwoodi* no visible galls were formed on the roots of these cultivars. Similar results were found by Brinkman *et al*. (1996) who reported cultivars of bean as good hosts for *M. chitwoodi*, but non hosts for *M. fallax*. Selected cultivars of bean can be a good choice in crop rotations for *M. fallax* infested fields.

The temperate root-knot nematodes *M. chitwoodi*, *M. fallax* and *M. hapla* are present in Belgian fields, alone or as mixed populations (Wesemael, unpublished data). The differences we found in host plant status of bean cultivars for these *Meloidogyne* spp. stress the importance of a correct diagnosis of the nematode species in agricultural fields.

Chapter 8

General discussion

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The polyphagous root-knot nematode *M. chitwoodi* causes severe damage in economically important crops such as potato, black salsify and carrot. Severe galling induces a rough surface, rendering infected crops unprocessable for the canning industry. The triage of damaged crops increases the costs and, therefore, if the percentage damaged crops as detected through pre-harvest quality control is too high, sometimes crops are not harvested but destroyed. When a farmer is confronted with this, there is a strong temptation to disinfect the field by chemical means. However, chemical treatment of the soil does not kill all of the nematodes (Hague & Gowen, 1987; Hooper & Evans, 1993) and, as *M. chitwoodi* can have multiple generations during one crop growing season (Pinkerton *et al*., 1991), chemical treatments might even be completely unsuccessful. Moreover, the effect of nematicides depends on the depth of application (Heald, 1987). As *M. chitwoodi* can be present in deeper soil layers (see chapter 5), most likely these nematodes would not be affected by chemical soil treatments. I could not conclude that migration to shallow layers took place, but damage to crops caused by upwardly migrating J2 of *M. chitwoodi* was reported in tomato (Pinkerton *et al*., 1987) and potato (Mojtahedi *et al*., 1991). Moreover, in the case of black salsify, the harvested tap root can reach lengths of up to 30 cm and more, and the presence of *M. chitwoodi* in deeper soil layers can be a potential risk for quality damage. These findings, together with environmental concerns, should encourage the farmer to reconsider the use of nematicides.

If the use of nematicides is discouraged, what other options does the farmer have? First of all it is of major importance that infested fields are detected before use. To avoid losses, in general no carrots or black salsify will be scheduled if *M. chitwoodi* is present in a field. In Belgium, the detection of *M. chitwoodi* mainly depends on extensive soil sampling. The soil sampling is extensive to reduce the costs of sampling and the subsequent diagnosis. The chances that nematode infestations will be detected with these sampling practices decrease with low population densities. The results presented in chapter 5 show that the greatest numbers of *M. chitwoodi* were found immediately after harvest for summer barley and carrot. In fodder beet, a crop with a long field period, the population of *M. chitwoodi* started to decline before harvest. Therefore, I would strongly suggest sampling immediately after harvest.

Detection chances of *M. chitwoodi* can be improved by incubation. Incubation allows juveniles to hatch from eggs present in the soil or in the organic material. The success of incubation strongly depends on the hatching physiology of J2. *Meloidogyne chitwoodi*

overwinters as eggs from which the J2 hatch as soil temperatures increase in the spring (Pinkerton *et al*., 1991). However, as shown in chapter 4 with *M. chitwoodi* egg masses collected from tomato plants, the hatching behaviour alters with the age of the host plant. A delay in hatching was observed when egg masses were collected from young plants compared with hatching of juveniles from egg masses collected on senescing plants. Moreover, I found that a vast amount of J2 might be in a state of obligate quiescence when egg masses were taken from senescing tomato plants. These J2 required root diffusates for substantial hatch. Therefore, the use of incubation is complicated, time consuming and undoubtedly will raise the cost of diagnosis.

Another option to increase the detection changes is adapting the depth of sampling cores to the vertical distribution of *M. chitwoodi* in the field. Root-knot nematodes have greater winter survival at deep rather than at shallow soil profiles (Nusbaum, 1962; Starr & Jeger, 1985). The results presented in chapter 5 suggest that the vertical distribution of *M. chitwoodi* is persistent in crop rotations and depending on field characteristics.

The best solution to increase the detection changes of even low densities of *M. chitwoodi* is to intensify the soil sampling. As shown in chapter 6 small infestations of *M. chitwoodi* can induce an important degree of damage and huge population build ups. Therefore, it is important to be able to detect small infestations. Moreover, phytosanitary measures linked to the quarantine status of *M. chitwoodi* require a fast detection of infestations. Intensifying sampling procedures increases the costs but provides better estimates of the actual population densities, allowing improved management. Recently a decision support system, NemaDecide, has been developed in The Netherlands for the management of potato cyst nematodes (Been *et al*., 2005). If possible this system will be adapted for *Pratylenchus* spp. and *Meloidogyne* spp. (Been, pers. comm.) and should provide farmers with predictions about possible yield losses and population development in time.

The initial higher costs of more intense sampling and subsequent analysis of soil samples can be compensated by less frequent soil sampling. I found that *M. chitwoodi* was still present in the field after several months without a host (chapter 5). Therefore, annual soil sampling for the detection of *M. chitwoodi* is not required once a population is detected. However, in Belgium the majority of farmers work with leaseholds and these can change annually. This might discourage farmers to invest in soil sampling. I suggest that the sampling history and nematode data of fields should be recorded in a field logbook or

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passport. These data can be combined with the rotation history of the field and can be a guide when both farmers and the canning industry have to make decisions on crop rotations. Moreover, a clear view on problem fields may help to prevent further spread of root-knot nematodes. Farm machinery or vehicles can spread nematodes through adhering infested soil particles; unfortunately cleaning of machinery is generally not practised. If infested fields are localised, field practices on these fields can be grouped and before the machinery is used on *Meloidogyne*-free fields they can be cleaned thoroughly. The same strategy should be used when crops are harvested. It is clear it will only be possible to schedule field practices on infested fields together if the distance between them is limited.

At present, different sampling procedures and diagnostic procedures are used to determine nematode population densities. Laboratory errors add to the variability of population densities found in the soil. For *Meloidogyne* spp. Schomaker *et al.* (2006) found a laboratory error of more than 50% when sub samples from well mixed bulk samples were processed with two different extraction techniques. To allow for a correct use of these data, procedures should be standardized.

An important obstacle for the registration of *Meloidogyne* infested fields is the quarantine status of *M. chitwoodi* and *M. fallax*. In fact, farmers fear the phytosanitary measures linked with this status more than the nematode itself. Therefore, a database with field information regarding the presence of these pests will only be possible if solid agreements with the authorities are made. One might ask if the quarantine status of *M. chitwoodi* should be reconsidered. The quarantine status might help to find grounds for research but it makes it more difficult to find farmers who are willing to collaborate.

Due to the wide host range of *M. chitwoodi* (Santo *et al.*, 1980; O'Bannon *et al.*, 1982; Ferris *et al.*, 1993; den Nijs *et al.*, 2004) it is very difficult to reduce its population densities by means of crop rotation. The succession of bean cv. Polder and marigold *Tagetes patula* cv. Single Gold decreases the population of *M. chitwoodi* substantially (see chapter 5). However, the experiments in chapter 7 show that *M. chitwoodi* is able to reproduce on bean cv. Polder. Compared with other cultivars, egg mass formation was delayed on cv. Polder. Most likely, the development of *M. chitwoodi* will be slower under field conditions than under the controlled conditions described in chapter 7. Therefore, bean cv. Polder could be an option for crop rotations. Nematode densities up to 1000 J2/ 100 g soil do not have detrimental effects on the vegetative growth of bean (chapter 7) and a normal harvest might be expected. The growing of bean, a crop with a short field period,

allows marigold to be grown later in summer. The population density of *M. chitwoodi* decreases under marigolds (chapter 5). Ploeg (1999) reports suppressed galling and reproduction of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* on tomato grown after marigolds. A reduction of population densities of *Pratylenchus* spp. by 70 to 95% after 3 months under marigolds has been reported by Lung *et al*. (1997). More recently Pudasaini *et al*. (2006) found a persistent effect of marigold on *P. penetrans* population densities during two crop cycles of host plants. I recommend marigold in crop rotations when *Meloidogyne* and *Pratylenchus* are concurrent species in a field. To control *P. penetrans* effectively, a monoculture of marigold for at least three months is required (Kimpinski *et al*., 2000; Evenhuis *et al*., 2004). When beans were harvested at the end of July or early August, the remaining summer period might be too short for marigold. In this situation, the farmer would not want to lose a full year because of the lack of a marketable crop, so early potatoes might be an option. Brommer and Molendijk (2001) report limited damage caused by *M. chitwoodi* in early potatoes, which are harvested at the end of June to the beginning of July.

During winter, under black fallow, the population of *M. chitwoodi* decreases substantially (chapter 5). This was also reported by Pinkerton *et al*. (1991). However, in view of possible erosion, farmers might prefer to grow cover crops. Moreover, European policy tends to phase out support for fallow thus compromising a major strategy for control of *M. chitwoodi* infestations. At present commercial cultivars of fodder radish with marked resistance against *M. chitwoodi* are marketed, although cultivars with complete resistance are not yet available (Korthals *et al*., 2006). Growing rapeseed for two months and incorporating the plants into the soil as a green manure resulted in a greater reduction of the *M. chitwoodi* population than under fallow (Mojtahedi *et al*., 1991). Green manure treatments increased yield of potatoes by 106-185% and reduced *M. chitwoodi* tuber infection compared to fallow in greenhouse and field microplots (Al-Rehiayani & Hafez, 1998).

Crop rotations can promote shifts in nematode populations (Johnson *et al*., 1996). The screening results for *M. chitwoodi*, *M. fallax* and *M. hapla* on different cultivars of bean (chapter 7) suggest that *M. hapla* populations would increase significantly under bean. Therefore, decisions on crop rotations should not only be based on the presence of *M. chitwoodi*.

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Further considerations

To be able to develop sound management strategies for *M. chitwoodi*, the knowledge currently available should be combined in decision making programs. However, at present different procedures are used, both in research and in diagnostics. This complicates assessments and might lead to wrong conclusions. Therefore standardized methods should be discussed and developed.

In 2004 a new *Meloidogyne* species, *M. minor* was detected on potato. Our experience and knowledge with *M. chitwoodi* should help us to avoid serious problems with this new pest.

Global warming most likely will allow *M. chitwoodi* to spread to regions more to the north and to have more generations per season and, therefore, increase problems. Moreover, the outbreak of tropical *Meloidogyne* species from glasshouses becomes no longer hypothetical. Rather than investing in research on the potential risk of these pests in temperate agriculture, we should take this for granted and invest in solutions.

Summary

Root-knot nematodes are classified within the genus *Meloidogyne* Göldi, 1892 and are found worldwide. They are obligate plant pathogens and parasitize nearly every species of higher plants. In Belgium, the root-knot nematode *Meloidogyne chitwoodi* is a major problem in the culture of field vegetables for the food canning industry on sandy soils in the provinces Antwerp and Limburg. *Meloidogyne chitwoodi* has a wide host range and can have multiple generations during a crop-growing season. The nematode causes severe damage to economically important crops such as potato, black salsify and carrot. In 1998 *M. chitwoodi* was listed as quarantine pest in the EU. The aim of this study was to increase the knowledge on the biology of *M. chitwoodi* in relation to crop rotations with field vegetables, and to increase the detection changes of this soil borne pest.

Comparative studies were made on the effects of root diffusates and host age on the *in vitro* hatching of *M. chitwoodi* and *M. fallax*. There is a marked contrast in the hatching response of the two species. Hatching of second-stage juveniles (J2) of *M. chitwoodi* produced on young plants did not require host root diffusate stimulus, whereas at the end of the plant growing season, egg masses contained a percentage of unhatched J2 that require host root diffusate to cause hatch. This form of obligate quiescence at the end of the host growing season was not found in *M. fallax*. This species hatched well in water and did not require hatch stimulation from root diffusate, irrespective of the age of the plant on which the egg masses were produced. The number of eggs per egg mass for *M. fallax* collected on senescing plants was significantly greater than the number of eggs per egg mass for *M. chitwoodi*. The number of eggs per egg mass of *M. chitwoodi* decreased with plant age. The results are discussed in the context of the differing survival strategies of the two species.

Pre-planting soil sampling to detect *M. chitwoodi*-infestations supports farmers when taking decisions on the crop rotation. To develop an adequate sampling strategy, the vertical distribution of *M. chitwoodi* was examined under summer barley, carrot, fodder beet, bean, marigold and black fallow on two fields with a sandy soil. Soil samples were collected at monthly interval from April 2004 until April 2006. Cores were taken to a depth of 70 cm and split into 10 cm segments. Nematodes were extracted by zonal centrifugation. Fodder beet increased the population of *M. chitwoodi* immensely and also carrot was a good host. Barley was a moderate host and under bean and marigolds the population

decreased. The relative distribution of *M. chitwoodi* over the different soil layers during two successive years was consistent in each field. The different successions with good, moderate and poor hosts did not influence this distribution significantly. A logistic model was fitted to the mean cumulative percentages of nematodes at increasing soil depth. Farmers are advised to take soil samples for detection of *M. chitwoodi* immediately after harvest, especially after crops with a long field period. Adapting the depth of the cores taken to the vertical distribution of the population can increase the detection chance. The results suggest that this distribution is persistent in crop rotations and depending on field characteristics.

Nineteen carrot cultivars were screened for potential resistance for *M. chitwoodi*. Egg masses of *M. chitwoodi* were found on all cultivars. However, there were no egg masses on more than 80% of plants of cvs Berlanda, Bolero, Chantenay, Nantucket and Parmex. By contrast, on cvs ABK, Douceur, Maxi and Merida egg masses were formed on all of the tested plants. To gain information about the damage caused by *M. chitwoodi*, carrots were grown in soil infested with different densities of nematodes. There was no effect of *M. chitwoodi* on the length, width and weight of the carrot taproot. Damage caused by *M. chitwoodi* was manifested by severe galling near the lenticels. Inoculation of nematodes 6 weeks after the carrots emerged resulted in a higher percentage of infected carrots and damaged taproots compared with earlier inoculation times. The effect of the time of harvest on nematode infection and damage was examined. When harvested 100 days after sowing in soil with low nematode densities (J2/ 100 g soil), no damage was reported. Harvesting 120 and 140 days after sowing resulted in 10% and 20% damaged carrots, respectively. With initial *M. chitwoodi* densities of 25 J2/ 100 g soil, the percentage of damaged taproots increased from 10% when harvested 100 days after sowing to 70% when harvested 140 days after sowing. In a field trial 11.5% of the carrots were damaged after a field period of 139 days and the initial *M. chitwoodi* population increased from 3 to 111 J2/ 100 g soil. It is recommended that growing carrots in *M. chitwoodi* infested fields should be avoided. However, damage can be limited in fields with low initial nematode populations when the period before harvest is reduced.

The host suitability of different bean cultivars was studied based on the ability of *M. chitwoodi* to develop inside bean roots and the production of egg masses. The production of egg masses of *M. chitwoodi* on bean was compared with egg mass production of two other temperate root-knot nematodes, *M. fallax* and *M. hapla*. The tested cultivars of bean

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were poor to good or even excellent hosts for *M. chitwoodi*, non hosts or poor host for *M. fallax* and excellent hosts for *M. hapla*. The differences found in host plant status between these three nematode species stress the importance of a correct diagnosis of the nematode species in agricultural fields. It seemed that, compared with other cultivars, the formation of *M. chitwoodi*-egg masses on cv. Polder was delayed. The development of *M. chitwoodi* inside the roots of four bean cvs was monitored. After 6 weeks only females were found inside the roots, indicating that *M. chitwoodi* was able to develop in all of the four tested cultivars. There was no difference between the cultivars.

Samenvatting

Wortelknobbelnematoden behoren tot het genus *Meloidogyne* Göldi, 1892. Het zijn obligaat plantenparasieten en ze worden wereldwijd aangetroffen. In België zorgt de maïswortelknobbelnematode *Meloidogyne chitwoodi* voor ernstige problemen in de vollegrondsgroenteteelt voor de conservenindustrie op de zandgronden van de provincies Antwerpen en Limburg. *Meloidogyne chitwoodi* kan zich vermeerderen op een groot aantal planten waaronder vele land- en tuinbouwteelten. Bij vele veroorzaakt *M. chitwoodi* schade. De meest spectaculaire schade treedt op bij aardappelen, wortelen en schorseneren die volledig waardeloos worden. Sinds 1998 is *M. chitwoodi* een quarantaineorganisme binnen de EU. Het doel van deze studie was de kennis over de biologie en de detectiekans van *M. chitwoodi* in relatie met teeltrotaties in de grove groenteteelt uit te breiden. Er werd getracht een strategie te ontwikkelen om problemen met deze nematode te beperken.

In vitro werd een vergelijkende studie gedaan over de invloed van wortelexudaten en de ouderdom van de waardplant op het uitkomen van juvenielen uit eieren van *M. chitwoodi* en *M. fallax*. Er was een duidelijk verschil tussen beide nematodensoorten in het uitkomen van juvenielen. Het uitkomen van tweedestadiumjuvenielen (J2) van *M. chitwoodi* uit eimassa's afkomstig van jonge, actief groeiende planten gebeurde spontaan en werd niet beïnvloed door wortelexudaten. Echter, eimassa's van planten aan het einde van het groeiseizoen bevatten een percentage juvenielen die enkel uit kwamen in aanwezigheid van wortelexudaten van de waardplant. Deze vorm van obligate quiescentie aan het einde van het groeiseizoen van de waardplant werd niet vastgesteld bij *M. fallax*. Juvenielen van *M. fallax* kwamen spontaan uit in water en er was geen stimulatie nodig van wortelexudaten onafhankelijk van de ouderdom van de waardplant waarop de eimassa's werden gevormd. Het aantal eitjes per eimassa van *M. fallax* verzameld op afstervende planten was significant hoger dan het aantal eitjes per eimassa van *M. chitwoodi*. Het aantal eitjes per eimassa van *M. chitwoodi* daalde met de ouderdom van de waardplant. De verschillen die werden opgetekend tussen *M. chitwoodi* en *M. fallax* wijzen mogelijk op een verschillende overlevingsstrategie tussen beide nematodensoorten.

Bemonsteringen en bodemanalyses om besmettingen met *M. chitwoodi* op te sporen helpen de telers bij het plannen van gewasrotaties. Op *M. chitwoodi* besmette percelen wordt geen contractteelt voor wortelen en schorseneren uitgevoerd. Om de toe te passen bemonsteringstrategie te verbeteren werd de verticale distributie van *M. chitwoodi*

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onderzocht in teeltrotaties met zomergerst, wortel, voederbiet, boon, afrikaantjes en zwarte braak. Op twee natuurlijk *M. chitwoodi*-besmette percelen werden vanaf april 2004 tot april 2006 maandelijks bodemmonsters verzameld. Bodemmonsters werden getrokken tot op 70 cm diepte en verdeeld in segmenten van 10 cm. Per bodemlaag werden de nematoden geëxtraheerd met zonale centrifuge. De populatie van *M. chitwoodi* nam enorm toe onder voederbiet en ook wortel was een zeer goede waardplant. Zomergerst was een matige waardplant en onder boon en afrikaantjes daalde de populatie van *M. chitwoodi*. De relatieve verdeling van *M. chitwoodi* over de verschillende bodemlagen was consistent in beide percelen gedurende de twee opeenvolgende jaren van onderzoek. De rotaties met goede, matige en niet-waardplanten had geen significant effect op deze verdeling. Er werd een logistisch model berekend voor de gemiddelde cumulatieve nematoden percentages bij toenemende bodemdiepte. Er wordt aangeraden om bodembemonsteringen voor de detectie van *M. chitwoodi* uit te voeren onmiddellijk na de oogst, zeker bij gewassen met een lange veldperiode. De bemonsteringsdiepte aanpassen aan de verticale verdeling van de *M. chitwoodi*-populatie verhoogt de detectiekans. De resultaten doen vermoeden dat deze verdeling persistent is in teeltrotaties en afhangt van veldkarakteristieken.

Negentien wortel cultivars werden onderzocht op aanwezigheid van resistentie voor *M. chitwoodi*. Eimassa's van *M. chitwoodi* werden gevonden op alle geteste cultivars. Echter, op meer dan 80% van de planten van cultivars Berlanda, Bolero, Chantenay, Nantucket en Parmex werden geen eimassa's waargenomen. Daarentegen werden bij cultivars ABK, Douceur, Maxi en Merida eimassa's gevonden op alle planten. Om de schade die wordt veroorzaakt door *M. chitwoodi* te onderzoeken werden wortelen geteeld in grond besmet met verschillende dichtheden van deze nematode. Er was geen effect van de *M. chitwoodi* besmetting op de lengte, de dikte en het gewicht van de penwortel. De schade veroorzaakt door *M. chitwoodi* manifesteerde zich voornamelijk in de nabijheid van de lenticellen. Planten die werden geïnoculeerd 6 weken na opkomst vertoonden een hoger percentage infectie en ondervonden meer schade door *M. chitwoodi* in vergelijking met inoculaties op vroegere tijdstippen. De invloed van de duur van de veldperiode van wortel op de infectie met *M. chitwoodi* en de veroorzaakte schade werden onderzocht. Wanneer wortelen werden geoogst 100 dagen nadat ze waren gezaaid in besmette grond met lage nematodendichtheden (2 J2 per 100 g grond) werd geen schade vastgesteld. Bij oogst 120 en 140 dagen na het zaaitijdstip werd respectievelijk 10% en 20% schade opgetekend. Bij een nematodendichtheid van 25 J2 per 100 g grond steeg de schade van 10% bij oogst 100

dagen na zaaien tot 70% bij oogst na 140 dagen. In een veldproef werd na een veldperiode van 139 dagen 11,5% schade vastgesteld en de initiële nematodenpopulatie steeg van 3 J2 per 100 g grond tot 111 J2/ 100 g grond. De teelt van wortelen in *M. chitwoodi* besmette grond wordt afgeraden maar schade kan worden beperkt door een verkorting van de veldperiode.

De waardplantgeschiktheid van verschillende bonencultivars voor *M. chitwoodi* werd onderzocht op basis van de ontwikkeling van deze nematode in bonenwortels en de vorming van eimassa's. De vorming van eimassa's van *M. chitwoodi* op boon werd vergeleken met de ontwikkeling van eimassa's van twee andere wortelknobbelaaltjes, *M. fallax* en *M. hapla*. De geteste bonencultivars waren slechte tot goede en zelfs zeer goede waardplanten voor *M. chitwoodi*, niet-waardplanten of slechte waardplanten voor *M. fallax* en zeer goede waardplanten voor *M. hapla*. De verschillen die werden gevonden in waardplantstatus voor deze drie nematodensoorten toont het belang aan van een correcte diagnose van de nematodensoort in landbouwpercelen. In vergelijking met andere bonencultivars bleek de vorming van eimassa's op cultivar Polder trager te verlopen. De ontwikkeling van *M. chitwoodi* in de wortels van vier cultivars werd onderzocht. Zes weken na inoculatie met *M. chitwoodi* werden enkel vrouwtjes teruggevonden in de bonenwortels. *Meloidogyne chitwoodi* was in staat om zich te ontwikkelen in elk van de vier cultivars en er werd geen verschil vastgesteld tussen de cultivars.

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Publications

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Wesemael, W.M.L. & Moens, M. (2008). Quality damage on carrots (*Daucus carota* L.) caused by the root-knot nematode *Meloidogyne chitwoodi*. *Nematology* 10 (in press).

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Oral presentations

Wesemael, W., Perry, R.N. & Moens, M. Host plant influences on the hatching of the root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*. 18th Symposium of the Nematological Society of Southern Africa, Port Elizabeth, South Africa, 9-10 May 2007.

Wesemael, W. Populatiedynamiek van *Meloidogyne chitwoodi* in teeltrotaties in de vollegrondsgroenteteelt. *Meloidogyne* werkgroep, Plantenziektekundige dienst, Wageningen, 20 March 2007.

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