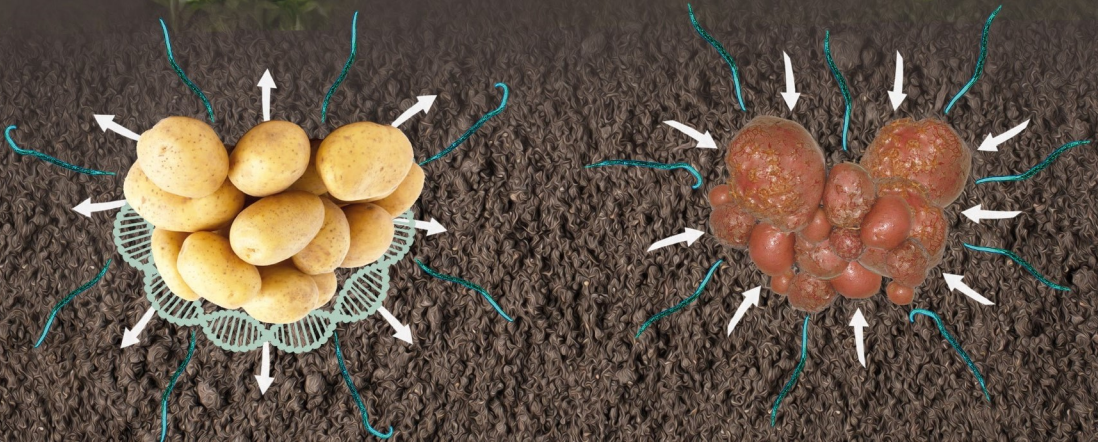


Quantitative studies on potato genotypes and fodder radish varieties resistant to the root-knot nematode *Meloidogyne chitwoodi*

Misghina Goitom Teklu



Propositions

1. The central dogma stating that multiple generations per growing season result in higher maximum population densities of plant-parasitic nematodes than single generations needs to be revised. (*This thesis*)
2. Conclusive evidence for true zero counts of nematodes, originating from a well-mixed soil sub-sample, cannot be established. (*This thesis*).
3. A hodgepodge of loose facts and preconceived ideas that are embedded in the literature eventually deter investigative research.
4. Due to the finite nature of data collection, simulations are contributory to broaden the spectrum of our understandings.
5. Societal reaction to scientific breakthrough is the same in both the developed and undeveloped world: suspicion always comes first.
6. The Dutch understood that aggressive nematode control strategies to eradicate plant-parasitic nematodes do not succeed and they correctly chose to live with them.

Propositions belonging to the thesis, entitled
Quantitative studies on potato genotypes and fodder radish varieties
resistant to the root-knot nematode *Meloidogyne chitwoodi*

Misghina Goitom Teklu
Wageningen, 8 May, 2018

**Quantitative studies on potato genotypes and fodder
radish varieties resistant to the root-knot nematode
*Meloidogyne chitwoodi***

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**Quantitative studies on potato genotypes and fodder
radish varieties resistant to the root-knot nematode
*Meloidogyne chitwoodi***

MISGHINA GOITOM TEKLU

Thesis

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Prof. Dr A.P.J Mol,

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Chapter 1

General introduction

General introduction

Potato (*Solanum tuberosum* ssp. *tuberosum*) is a major food crop worldwide. In total, 382 million tonnes were produced in 2014 (FAOSTAT, 2017). The Netherlands is a major producer of ware, starch and seed potatoes ranking in the top ten of potato producing countries. According to Statistics Netherlands (CBS, 2017), 654,309 ha arable land was cultivated in 2017, of which 162,610 ha, ca. 25%, was under potato cultivation (Figure 1.1). The largest proportion of Dutch potato production consists of ware potatoes (ca. 50%), followed by starch potatoes (36%) and seed potatoes (14%). About 15% of the ware potatoes is utilized for direct consumption and 85% is processed (Haverkort, 2015).

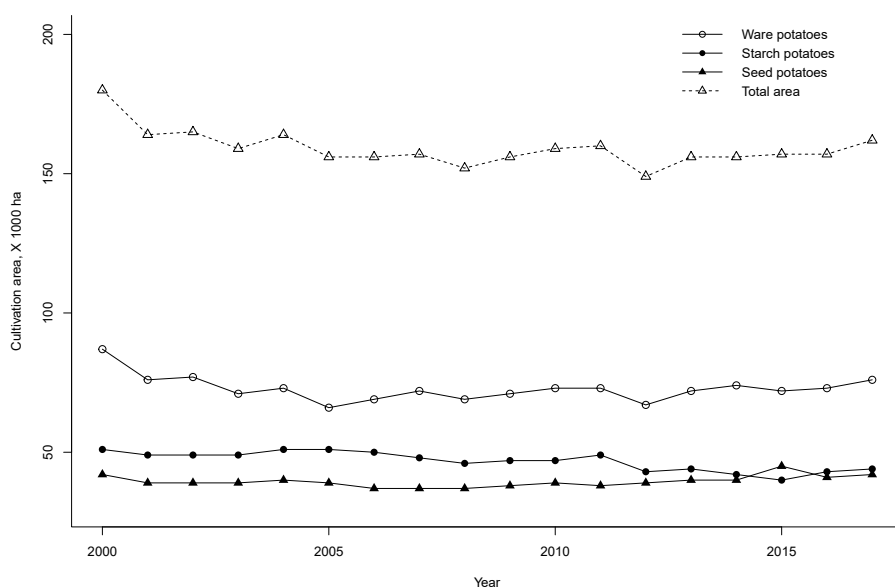


Figure 1.1. Total area of arable land for potato production in The Netherlands from 2000 - 2017, as well as the area used for ware, starch and seed potatoes (CBS, 2017). Data accessed on 29-11-2017.

Dutch potato growers are at the top in terms of production per ha and export of high-quality seed potatoes. According to The Dutch Organization for Potato Merchants (NAO), in 2015/2016 the Dutch seed potato sector covered 60% of the world market, exporting 800,000 tonnes (70% of the total seed potatoes produced) to 80 countries throughout the world, while 30% is used domestically within The Netherlands. In 2015 the area cultivated for seed potatoes increased with 12%, as compared to ware and starch potatoes, (CBS, 2017) (Figure 1.2).

Dutch potato cultivars are in demand worldwide, not only for their quality but also because of their resistance to *Phytophthora infestans* and Q-organisms, e.g. *Globodera* spp., the potato cyst nematodes (PCN), *Globodera rostochiensis* and *Globodera pallida*. The availability of resistant cultivars has led to a significant reduction of the use of soil fumigants in The

Netherlands against PCN, promoted higher yields for growers and conserved seed potato acreage for Dutch traders in The Netherlands (Been & Schomaker, 1998).

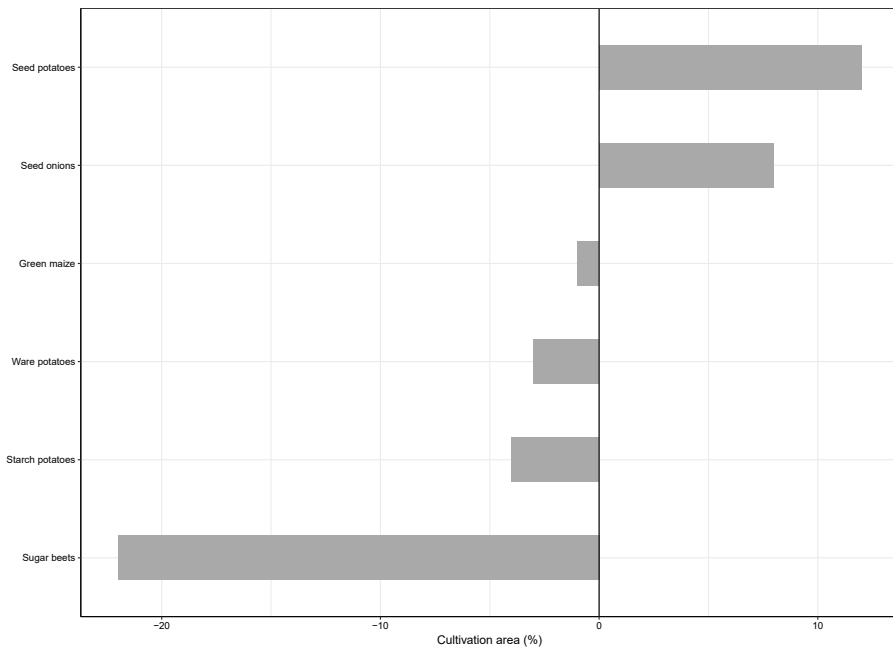


Figure 1.2. The increase in cultivation area (%) of seed potatoes as compared to ware and starch potatoes and other crops in The Netherlands in 2015 (CBS, 2017). Data accessed on 29-11-2017.

Besides the favourable Dutch climatic conditions, the high level of organization between farmers, growers, breeders, cooperatives and the utilization of research supported with updated science and technology in fields like agronomy, breeding, storage, transport, processing and marketing, are key to the success (Haverkort, 2015).

Plant-parasitic nematodes

The existence of plant-parasitic nematodes has been common knowledge for more than a century. More than 4000 species have been described so far, constituting approximately 15% of the total number of nematode species known (Decraemer & Hunt, 2013). They can cause financial losses both by reducing the quality (Figure 1.3) and/or the quantity of the produce harvested. According to Nicol *et al.* (2011) crop losses by plant-parasitic nematodes in the tropical and sub-tropical areas were estimated to be 14.6% compared to 8.8% in the temperate zone. Moreover, plant-parasitic nematodes can also expose a host to other pathogens such as fungi: *Verticillium* spp., *Fusarium oxysporium* and *Rhizoctonia solani* (Abawi & Chen, 1998; Rowe & Powelson, 2002; Back *et al.*, 2006). Virus vector nematodes, such as *Trichodorus* and *Paratrichodorus* spp (Decraemer & Geraert, 2013) are also of

concern. Although the impact of plant-parasitic nematodes can be severe, several species being included in the list of quarantine organisms in the EU (EC Directive 2000/29/EC) and EPPO region (A2 list N° 6.1 and 6.2), or the EPPO alert list (EPPO, 2008), data on the actual financial damage caused by plant-parasitic nematodes are difficult to obtain. According to Elling (2013) economic losses amount to \$173 billion worldwide.

Root-knot nematodes (RKN)

Among the plant-parasitic nematode genera, the genus *Meloidogyne*, the root-knot nematode, is the most widely distributed nematode genus in the world, capable of colonizing nearly every higher plant and able to cause economic damage (Karssen *et al.*, 2013). The description of *M. javanica*, *M. hapla*, *M. incognita*, and *M. arenaria* by Chitwood in 1949 initiated the recognition of the economic importance of root-knot nematodes (Taylor & Sasser, 1978). According to Hussey & Janssen (2002) they constitute more than 90% of the root-knot nematode infestations of arable land in the world and a major part of the economic losses estimated by Elling (2013) is attributed to this major group of root-knot nematodes. A recent survey by Jones *et al.* (2013) provides a number one ranking of root-knot nematodes in the top 10 list of plant-parasitic nematodes in molecular plant pathology based on scientific and economic importance.

A number of ‘emerging’ root-knot nematode species, *M. chitwoodi*, *M. fallax*, *M. minor*, *M. enterolobii*, *M. exigua*, and *M. paranaensis* found in Europe and the USA, are potentially able to cause damage in major arable crops, *e.g.* potato (Moens *et al.*, 2009; Elling, 2013). Currently, no damage estimates for Europe are available for root-knot nematodes in general (Wesemael *et al.*, 2011).

Meloidogyne chitwoodi and *Meloidogyne fallax*

Host range and distribution worldwide

The root-knot nematode *Meloidogyne chitwoodi* (Golden *et al.*, 1980) and its close sibling *Meloidogyne fallax* (Karssen, 1996) are considered as temperate nematodes. *Meloidogyne chitwoodi* is also commonly known as the Columbia root-nematode / maize root-knot nematode (Santo *et al.*, 1980) and *M. fallax* as the false Colombia root-knot nematode. These two nematodes were first described to be present in the EPPO region, The Netherlands, by Karssen (1995, 1996). Their polyphagous endo-parasitic nature enables them to colonize both mono- and dicotyledon plant species (O’Bannon *et al.*, 1982; Brinkman *et al.*, 1996; Kutnywayo & Been, 2006). Both nematodes can cause severe quality damage (Figure 1.3) to potato (*Solanum tuberosum*), carrots (*Daucus carota*) and black salsify (*Scorzonera hispanica*) (Wesemael & Moens, 2008; Norshie *et al.*, 2011; Heve *et al.*, 2015; EPPO, 2017). Because of the economic damage, primarily to potato, both nematodes are listed as quarantine nematodes in the United States of America, Canada and Mexico (Smith *et al.*, 1997). Since 1998, *M. chitwoodi* and *M. fallax* are also listed as quarantine pest in the EU.

Currently *M. chitwoodi* is distributed within and outside Europe (Den Nijs *et al.*, 2004; EPPO, 2017). It is now detected in Belgium, Portugal, Germany, Sweden, Turkey, France, North America including Mexico, South America (Argentina), South Africa, Mozambique, Australia and New Zealand (EPPO, 2017). *Meloidogyne fallax* is also detected in Belgium, France, England, Northern Ireland, Australia and New Zealand (EPPO, 2017).



Figure 1.3. Quality damage caused by *Meloidogyne chitwoodi* to three economically important crops **A)** Potato: deformation of the skin with heavy blisters. **B)** Carrot tap roots: stubby growth, forked, misshapen and hairy (Photos courtesy of Heve *et al.*, 2015). **C)** Black salsify: heavily galled with excessive growth of hairy roots (Photos courtesy of WUR-PAGV).

The situation in The Netherlands and the European Union (EU)

The concept of introducing quarantine measures is to prevent further spread of the pests within and, especially, outside the infected area. Various phytosanitary measures were implemented in The Netherlands to contain *M. chitwoodi* and *M. fallax*. These include general surveys (in all hosts) and specific surveys (potatoes), including checking 200 seed potato tubers after harvest and incubation for presence of the nematode in each lot, restriction of growing seed potatoes in a radius of 1 km around an infected site (Plant Protection Service, 2017). Contaminated areas are subject to containment with the objective of preventing further spread of the nematode (EPPO, 2013). In The Netherlands, ca. 425 findings of *M. chitwoodi* or *M. fallax* were reported from plant lots and propagation material in the period between 1995 and 2016 (Figure 1.4), the majority of which consisted of *M. chitwoodi* (Plant Protection Service, 2010 – 2017). This indicates that despite the quarantine regulations imposed, the number of infestations is still increasing.

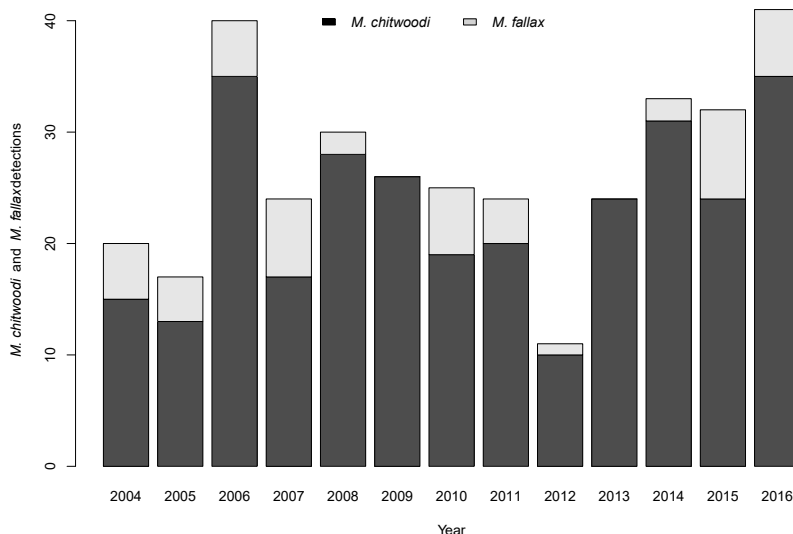


Figure 1.4. Detections of *Meloidogyne chitwoodi* and *M. fallax* from plant lots and propagation material in The Netherlands from 2004-2016 (Plant Protection Service, 2010-2017). Data accessed on 1-12-2017.

The pest risk analysis of *M. chitwoodi* identifies nine pathways for its local spread and long distance distribution (within the EU or beyond). Although there is only limited information available about the pathways, which also apply to *M. fallax* (Van der Gaag *et al.*, 2011; EPPO, 2013), the following four were considered the most important in distributing both nematodes: 1) planting host plants attached with or without soil originating from infested areas. 2) Planting of non-host plants with soil attached originating from infested areas. These two pathways include all propagation material except plants derived from tissue culture. 3) Tubers, bulbs and any other plant parts originating from infested areas and intended for consumption or processing. 4) Soil attached to or associated with tubers, bulbs and any other plant parts intended for consumption or processing originating from infested

areas. The last one does not account for local outbreaks as this refers to the long distance spread by transporting infested soil attached to plants (Van der Gaag *et al.*, 2011).

Pathway-1, the distribution by host plants intended for planting includes a wide range of plant species and various planting materials such as tubers, bulbs and rooted plants with or without soil attached. Because of the huge exchange of plant products within the EU, potato seed tubers forming the major part of the total trade volume, pathway-1 is considered as the number one risk of spread. As described by Van der Gaag *et al.* (2011), based on data obtained from Eurostat (2011), about 1.5 and 13 million tonnes of seed and ware potatoes, respectively, were traded in the years 2008-2010 from countries where *M. chitwoodi* and *M. fallax* were established or reported in transient. These countries were mainly Belgium, Germany, France, The Netherlands, Portugal and the United Kingdom. Moreover, ca. 430 thousand tonnes beetroot salads, salsify, radish turnips and carrots originating from those six countries were exchanged. Detailed trade of crops and ornamental plants, which are potential hosts of *M. chitwoodi* and *M. fallax* within the EU, are listed in Table 1.1.

Considering the following:

- i) The polyphagous nature of *M. chitwoodi* and *M. fallax*.
- ii) The high risk of spread of *M. chitwoodi* and *M. fallax* by ware, starch and seed potatoes and other potential hosts traded within the EU.
- iii) Their adaptation to a broad range of climatic conditions, ranging from cold winters to warm summers predominantly in coarse texture (sandy) soils (EPPO, 2017).

Table 1.1. Traded crops and plant products from countries where *Meloidogyne chitwoodi* and *M. fallax* are established or in transient (under eradication): Belgium, Germany, France, The Netherlands, Portugal and United Kingdom to the rest of the EU. **Ornamentals:** dormant bulbs, tubers, tuberous roots, corms, crowns and rhizomes: **Root crops:** beetroot, salsify, radishes and similar edible roots. Trees, shrubs, bushes and outdoor live plants are also including roots. Source: (Eurostat, 2011; adapted from Van der Gaag *et al.*, 2011).

N°	Crops traded	Amount (tonnes)
1	Seed potatoes	1,549,200
2	Ware and processing potatoes	12,374,000
3	Carrots and turnips	21,951
4	Edible salads and root crops	408,206
5	Ornamentals	162,055
6	Gladioli corms	14,682
7	Chicory and roots	43,421
8	Tulip bulbs	78,478
9	Trees, shrubs and bushes	641,925
10	Outdoor live plants	465,603

It can be conclude that these nematodes might already be more widely distributed in the EU than is currently known. It takes time for a new introduction to establish, build up and reach the detection level (Van der Gaag *et al.*, 2011).

Issues of host races and pathotypes of *Meloidogyne chitwoodi* and *Meloidogyne fallax*

In the USA, the concept of the differential host test is widely accepted as a means to determine intraspecific variation with in species of root-knot nematodes since its report by (Sasser, 1952). Based on the definition of a differential host, originally two races of *M. chitwoodi* were recognized: race 1 and race 2. Races 1 and 2 were classified using two different hosts: carrots (*Daucus carota*), cv. Red Cored Chantenay and Lucerne (*Medicago sativa*), cv. Thor (Mojtahedi *et al.*, 1988; Ferris *et al.*, 1994). Later on race 3 was also isolated from Tulelake, California and is characterised by its reproduction on *Solanum bulbocastanum*, something both race 1 and 2 are not able to do (Mojtahedi *et al.*, 1994). Nevertheless, race 3 was later on considered a pathotype 1 of race 2 as it multiplies on alfalfa (Brown *et al.*, 2009; Humphreys-Pereira & Elling, 2013). An isolate from Washington, which also overcomes the resistance on *Solanum bulbocastanum*, was designated as pathotype 1 of race 1 as it multiplies in carrots (Mojtahedi *et al.*, 2007; Humphreys-Pereira & Elling, 2013). A summary of the differential host races and pathotypes is given in Table 1.2.

Table 1.2. Details on host races and pathotypes of *Meloidogyne chitwoodi* based on differential hosts (Adapted from Brown *et al.*, 2009).

Population of <i>Meloidogyne chitwoodi</i>	Differential hosts					
	Susceptible Tomato	Potato : cv. Russet Burbank/ Desiree	Carrot: cv. Red Cored Chantenay	Alfalfa cv. Thor	<i>Solanum bulbocastanum</i> SB22	<i>Solanum fendleri</i> , <i>Solanum hougasii</i>
CRKN-1 (host race 1)	Yes	Yes	Yes	No	No	No *
CRKN-1 _(P1) (pathotype 1 of race 1)	Yes	Yes	Yes	No	Yes	No
CRKN-1 pathotype <i>S. bul.</i> 1,2,3/	Yes	Yes	Yes	No	Yes	Yes*
CRKN-2 (host race 2)	Yes	Yes	No	Yes	No	No
CRKN-2 _(P1) (pathotype 1 of race2)	Yes	Yes	No	Yes	Yes	No

* Population of *Meloidogyne chitwoodi* from The Netherlands.

Currently, no valid evidence exists on the presence of different races in The Netherlands. Van der Beek *et al.* (1999) analysed eight populations of *M. chitwoodi* using a differential host test and all turned out to be race 1. An artificial isolate selected as a mono-female line from The Netherlands, was named pathotype *S. bul.* 1, 2, 3/, after circumventing the resistance from *S. fendleri*, *S. hougasii* and *S. bulbocastanum* (Janssen *et al.*, 1998). No evidence of races and pathotypes for *M. fallax* has been found so far (Van der Beek & Poleij, 2008).

Management options for *Meloidogyne chitwoodi* and *Meloidogyne fallax* in The Netherlands

Quarantine diseases are a major threat to seed potato production in The Netherlands (Van Arendonk, 2012), among which potato cyst nematodes (PCN) and root-knot nematodes (*M. chitwoodi* and *M. fallax*) are the most important.

While a management system is available for PCN, effective management of *M. chitwoodi* is still in development, although some qualitative information can be found on www.aaltjesschema.nl (Molendijk & Korthals, 2005), which helps farmers to avoid unfavourable crop rotations. When designing a management system for *M. chitwoodi* we can look at the successful management system for PCN in The Netherlands.

The potato cyst nematode became a problem in The Netherlands directly after the World War-II. It lasted until 1960 before a first integrated control approach was implemented (Hijink, 1972). The approach included crop rotation, *G. rostochiensis* resistant potato cultivars and soil fumigation (Table 1.3).

Table 1.3. Crop rotation schemes to control PCN in an integrated approach using resistant crops and application of a fumigant*. Adapted from (Hijink, 1972, 1974). Resistant (Res) and susceptible (Sus) cultivars are always used alternatively.

Rotation scheme	1 st year	2 nd year	3 rd year	4 th year	*Fumigation
(1:4)	Potato	Non-host	Non-host	Non-host	No
(1:3)	Potato	Non-host	Non-host	Potato	Before planting
(1:3)	Res. potato	Non-host	Non-host	Res/Sus. potato	No
(1:2)	Res. potato	Non-host	Res. potato	Non-host	Every 4 th year
(1:2)	Res. potato	Non-host	Sus. potato	Non-host	Every 4 th year

*Fumigation with either 250 liter 1,3-,dichloropropene (DD) or 400 liter ha⁻¹ Metam Sodium 70% before planting.

The system only gave short relief, but did not solve the problem. Problems just kept increasing because of the presence of *G. pallida*, the ineffectiveness of soil fumigation (Been & Schomaker, 1998) and the blind use of a standard crop rotation for nematode management. A new integrated approach was required and became feasible after the following four points were addressed:

- i) An intensive soil sampling system with known accuracy for seed, ware and starch potatoes was implemented.
- ii) The concept and use of (partial) resistance, expressed as relative susceptibility, against *G. pallida* was described (Phillips, 1984; Seinhorst, 1984) and implemented.
- iii) The Seinhorst models for the prediction of population development and yield loss, needed to evaluate crop rotations quantitatively, control measures (nematicides, trap crops, etc.), and degree of resistance, were applied.
- iv) Decision support systems (DSS) became available to enhance knowledge transfer and enable scenario studies of control strategies (www.nemadecide.com).

This provided the possibility to generate tailor made solutions for individual farmers instead of a general approach that will not fit every farmer. As a result, the amount of fumigants used in The Netherlands was reduced by more than 70% in early 2000 and the number and intensity of infestations was significantly reduced (Been & Schomaker, 1998; Been *et al.*, 2014; Been, 2015). Currently PCN is not a major problem in The Netherlands.

When we look at the root-knot nematodes *M. chitwoodi* and *M. fallax* Step 1, the intensive sampling techniques have been in development since 2003 (Been *et al.*, 2007). An early detection method for *M. chitwoodi* has been developed recently and was introduced in 2016 for farmers, use in The Netherlands. Step 3, the models for yield and quality loss, as for the population dynamics, are available and tested in the last decennium. A DSS (Step 4), incorporating *M. chitwoodi* and *Pratylenchus penetrans* is already available since 2010 and can be extended whenever new information becomes available (Been *et al.*, 2010).

What is dearly needed is information about chemical control, the population dynamic parameters of hosts and, most important, the availability of resistant arable crops.

Prospective of chemical control strategies

What we know so far is that still *M. chitwoodi* and *M. fallax* are far more vulnerable to chemical treatment (Molendijk & Mulder, 1996; Moens *et al.*, 2009; Elling, 2013; Viaene, 2014) than cyst nematodes. Although nematicides reduce populations of *M. chitwoodi*, it is unlikely that densities below the threshold level for quality damage will be attained (Griffin, 1985; Ingham *et al.*, 2000; Hafez & Sundararaj, 2002; Runia & Molendijk, 2007).

The other challenge is the reduction of the use of both nematicides and nematostats by EU-legislation. In 2005, as part of the Montreal protocol, the broad-spectrum fumigant methyl bromide was phased out due to its role in depletion of the ozone layer. Some are either banned *e.g.*, 1, 3 dichloropropene has been abandoned in 2008 (European Council Directive 91/414/EEC). Dazomet and Temik were withdrawn in 2011 and 2015, respectively from the EU. Others are drastically restricted *e.g.*, Monam (metam sodium) is no longer in use for potatoes since 2014. Regulations require that the product has to be placed at a minimum depth of 20 cm, the surface has to be sealed with virtually impermeable film for 14 days to avoid evaporation, a 150 m free zone has to be present around every ha fumigated and a buffer zone around the cadastral boundary of dwellings has to be established. Basically, it makes this treatment no longer economically feasible for any arable crop. As the remaining nematostats *e.g.* (Vydate, Mocap and Nemathorin) only reduce yield losses, but do not kill nematodes, their usefulness against *M. chitwoodi* is unclear (Runia *et al.*, 2006; Van Gastel-Topper *et al.*, 2009). With the current tendency to phase out the use of pesticides completely, it is not likely that chemical crop protection will be a major part of a future management system for *M. chitwoodi*.

Meanwhile, the drastic decline of soil fumigation in the last decades, used to control PCN, is now attributed to be one of the major reasons of the emergence of *M. chitwoodi* and *M. fallax*.

Challenges with alternative control strategies and the way forward

As only few non-hosts are available, the conventional concept of crop rotation is severely crippled. The broad use of green manure crops, susceptible to *M. chitwoodi*, as a winter cover, to prevent leaching of nitrogen from the soil, wind erosion and supply extra organic matter, enhanced the problems and is regarded as the second major cause of the increased presence of *M. chitwoodi* and *M. fallax*. Instead of the normal winter decline, 90% under fallow (Been *et al.*, 2007), populations are now maintained or even increase when a non-resistant green manure crop is used. Crop rotation, as in PCN, where only once in three to six years a host is grown, is not possible for *M. chitwoodi* and *M. fallax* due to the wide host-range. Only a few crops are reported as non-hosts or poor hosts, which including French beans, spinach and some green manure crops like *Tagetes patula* and fodder radish, (www.aaltjesschema.nl).

Therefore, future management of root-knot nematodes will be based on crop rotation with non-hosts, the use of poor hosts, and resistant crops. The information concerning the host status of many crops, currently regarded as hosts of *M. chitwoodi* is uncertain. Many of the data available are based on the density and environment dependent P_i/P_1 ratio. Thorough research on the host status might reveal that many crops, now designated as moderate or good hosts, are in fact bad hosts (Figure 1.5), as was found for carrots (Heve *et al.*, 2015) and susceptible fodder radish varieties (this thesis). Correct estimation of the host status, based on population dynamic models, will therefore be crucial for a management system of *M. chitwoodi*.

However, most important will be the development and subsequent screening of resistant crops, particularly those with a high economic impact (Boerma & Hussey, 1992). Resistance in potato genotypes differs from resistance in a green manure crop like fodder radish. In potato, resistance is obtained by introgression of resistant genes from wild and primitive tuber forming potatoes and as all potatoes are clones, the same genetic makeup applies to all plants. Primitive forms of cultivated potato and their wild relatives are considered as a useful source of traits in potato breeding (Brown, 2011; Machida-Hirano, 2015). Exploiting these genetic resources is practiced for more than a century (Hawkes, 1958). Machida-Hirano (2015), listed 65 wild and cultivated potatoes out of which more than 50% had traits with resistance to *M. incognita* and 32% against potato cyst nematodes. Some of these wild tubers were also sources of resistance to *M. chitwoodi* and *M. fallax* (Janssen *et al.* 1995, 1996, 1997; Norshie *et al.*, 2011).

Contrary to potato, resistance in fodder radish is achieved by a continuous selection process and cross breeding of seeds. At the end a certain, although high, fraction of the seeds will be resistant, but each seed is genetically different.

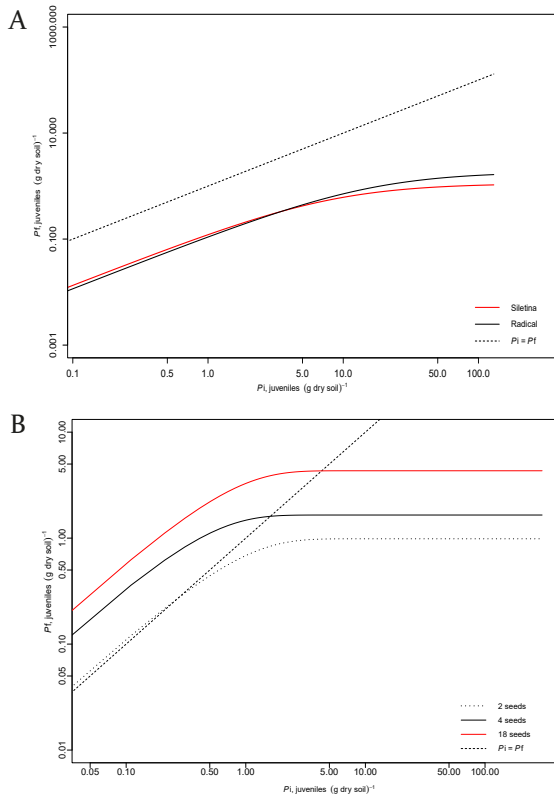


Figure 1.5. A) Based on population dynamic studies of *Meloidogyne chitwoodi*, two susceptible fodder radish varieties are in fact poor hosts: at each P_i the $P_f < P_i$. B) Carrot cv. Nerac, a 'moderate' host for *Meloidogyne chitwoodi* in fact turns out to be a poor host.

Resistant potato genotypes and Fodder radish varieties

In The Netherlands, research to identify resistant genes in wild and primitive cultivars of potato started in the nineties. Promising sources of resistance were obtained from *S. bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri* and *S. hougasii* (Janssen *et al.*, 1996). In 2000, the EU-funded project QLRT-1999-1462, DREAM (Durable Resistance Against *M. chitwoodi* and *M. fallax*) was started (Zoon *et al.*, 2002). The project focussed on:

- The identification and incorporation of resistance genes in arable crops like potato, carrots, black salsify and green manure crops (fodder radish and ryegrass etc.).
- The study of intraspecific-variation in virulence and durability of the resistance.
- The optimization of production systems by crop rotation.

Within a decade after the DREAM project ended, several breeding companies successfully produced potato genotypes with a single resistance gene against *M. chitwoodi* and possibly *M. fallax* (Draaistra, 2006). *Meloidogyne chitwoodi* resistant fodder radish varieties, also a result of the DREAM project, are already on the market since 2004 in Europe. One aspect that the project did not address however, is the establishment of a stable resistance measurement.

Commonly used resistance tests and their pitfalls

The quantitative estimation of the host-status and resistance of crops to plant-parasitic nematodes is a fundamental requirement for a management strategy. The lack of standard protocols for resistance testing became problematic since late 1980s, when resistance as means of control became more important. In fact, it was one of the major objectives of the international *Meloidogyne* consortium (1975-1984) by USAID to establish such a protocol for root-knot nematodes (Sasser *et al.*, 1984). A number of improvements were made since then: however, a method for wider application that can be repeated and applied at different locations providing the same results has not been adapted so far.

A large number of techniques to establish host-status / degree of resistance can be found in the literature and are used on a regular base. Two are used quite frequently when testing for resistance against *M. chitwoodi*. One is the multiplication rate or reproductive factor, ($R = P_f/P_i$), used to compare both different crops and different cultivars of one crop (Oostenbrink, 1966). A second method is the estimation of a root-galling index, based on scoring root knots of test plants in comparison with a susceptible reference plant. However, there are some severe drawbacks concerning these methods:

- i) *The multiplication rate P_f/P_i is not a stable factor.* Seinhorst in (1967, 1970) stated that; “many nematologist seem to be unaware of the possibility that the reproductive factor may not be independent of P_i ”. In fact, P_f/P_i is density dependent, which means the ratio changes with the initial population density (Figure 1.6). As nematodes, just like other organisms, compete with each other for space and food, here in the root system of the plant, the multiplication rate gets smaller when initial population densities get higher and competition increases. Even when the same P_i is used in different experiments, the multiplication rates will differ between experiments, due to differences in growing conditions and management.
- ii) *When the susceptible reference is not from the same species as the tested plant.* It is not uncommon that a susceptible tomato cultivar is used as a control when estimating the host-status of potato cultivars or green manure crops (Brown *et al.*, 1999; McSorley, 1999). This would imply that the population dynamic parameters of both crops would be comparable and that both crops can be compared at any density over the whole range of possible initial population densities. There is ample proof that this is not true. One should be aware that population development depends on the number of available feeding sites, that the size of the root system is limited and that every crop has a different root volume (Pudasaini, 2006), hence the larger the root system the higher the population density build up per unit soil.
- iii) It is known that galls can be empty and that some plant species and cultivars do not show galls while being infested *e.g.* fodder radish varieties (see Chapter 6). Moreover, the contents of a gall can vary extremely. In addition, just like the reproductive factor, there is a density dependency of the number of galls produced. In general, these points make the use of a gall index useless for management purposes.

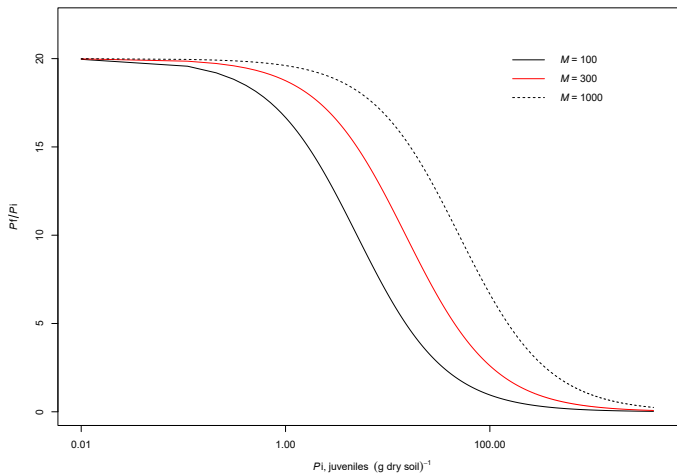


Figure 1.6. The density dependency of the reproductive factor, $R = (P_f/P_i)$ which is the ratio of final population (P_f) to the initial population density (P_i), to estimate host-status (Oostenbrink, 1966). Lines based on three theoretical maximum population densities: $M = 100$, 300 and 1000 J2(g dry soil) $^{-1}$.

The concepts of population dynamics in estimating host status and resistance

Based on the competition, every female of the root-knot nematode needs a certain amount of space in the root system to establish its feeding site. Based on the finite size of any root system, two important population dynamics parameters should be distinguished in the quantification of host suitability. The parameters have clear biological meaning (Seinhorst, 1966, 1967; Seinhorst *et al.*, 1995): the maximum multiplication rate (a) at very low initial nematode densities ($P_i \rightarrow 0$) when competition is absent, and the maximum population density (M) at high nematode density ($P_i \rightarrow \infty$), which is regarded as the carrying capacity of the root system of the investigated crop (Figure 1.7). The parameters are influenced by external conditions that change the size of the root system and are part of the models for population dynamics for both sedentary and migratory nematodes. They can only be estimated when using a range of population densities (Seinhorst, 1966, 1967, 1970). However, resistance, expressed as relative susceptibility (RS), when calculated from these parameters: $RSa = a_{res} / a_{sus}$ or $RSM = M_{res} / M_{sus}$ is independent of environmental factors as long as both the susceptible and tested host are grown under the same conditions (Phillips, 1984; Seinhorst, 1984). When RSa and RSM are the same, RS is density independent as was proven earlier for cultivars of potato and potato cyst nematodes and in this thesis for potato with *M. chitwoodi*.

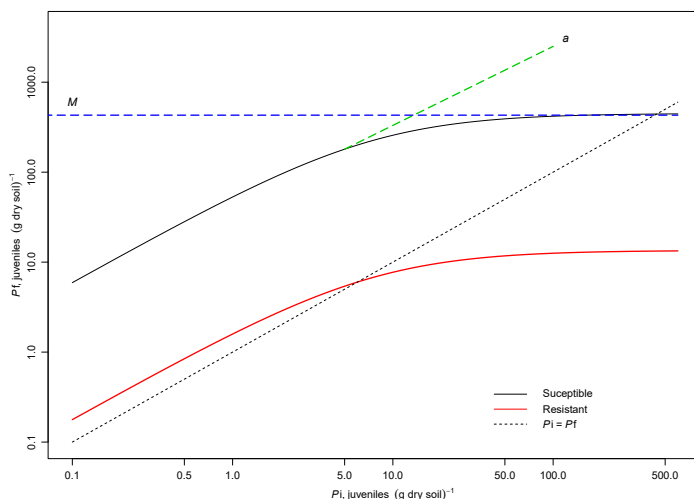


Figure 1.7. Theoretical lines according to equation: $P_f = M \times P_i / (P_i + M / a)$ describing the relation between P_i and P_f (log scale) of a susceptible and resistant host. Where: a is the maximum multiplication rate when $P_i \rightarrow 0$ and M is the maximum population density when $P_i \rightarrow \infty$. (After Seinhorst, 1967, 1970).

Pilot glasshouse experiments investigating the population dynamics confirmed the high resistance against *M. chitwoodi* of potato genotypes and fodder radish varieties (Norshie *et al.*, 2011; Teklu *et al.*, 2012, 2014). These findings resulted in the first “MeloResist” project, initiated by Wageningen University and the Dutch potato breeding companies aiming to evaluate resistance, estimate yield and quality damage of potato tubers and to develop a methodology for a routine resistance test. Breeders need a scientific, valid and economically feasible testing method to estimate the degree of resistance and, if possible, to get some information about yield and quality loss (tolerance). Farmers and advisory services need to know how these cultivars will perform in the field and how the nematode populations will develop. Therefore, the test needs to provide the parameter values required in an advisory system such as NemaDecide (Been *et al.*, 2005) and NemaDecide Geo plus (<https://akkerweb.nl/Shell>) used by farmers and extension services.

Scope of the thesis

The main objective of the research during the last seven years was to evaluate the degree of resistance of the newly developed *M. chitwoodi* resistant potato genotypes and fodder radish varieties and to develop a reliable and scientifically valid routine resistance test. The performance of these resistant crops was also evaluated using relevant yield and quality loss models. While this research was carried out, standard operating procedures and new techniques had to be developed and evaluated in order to be able to make progress. The current thesis, therefore provides a few sections of the work that has been conducted.

In **Chapter 2** a meta-analysis is presented on the data collected from four pot experiments on eight potato genotypes, seven with a resistance gene against *M. chitwoodi* and one for PCN under glasshouse conditions to study the population dynamics and to estimate resistance. The data were also used to evaluate the stability of their estimators in different pot sizes when downscaled from 10 to 5 to 3 and to 2 kg and finally to define a simple but reliable method for screening resistance of new potato genotypes.

Meloidogyne chitwoodi in potato is known for its quality damage; yield reduction is seldom. In **Chapter 3** a meta-analysis of the effect of the initial population density of *M. chitwoodi* on growth, fresh tuber weights and tuber quality of potato genotypes is performed. Tuber infestation levels of some potato genotypes after storage at 7°C for about 300 days were also studied as a pilot.

Effective management of *M. chitwoodi* under field conditions is realised when potato genotypes have resistance both in the roots and in the tubers. There are reports on the existence of two linked genetic factors controlling the inheritance of resistance to *M. chitwoodi* in roots and tubers in potatoes independently (Brown *et al.*, 2009). If this is true, as tuber formation starts later in the growing period of potato, only low nematode densities will be available to invade the tubers of resistant genotypes and tuber susceptibility might be overseen in pot tests. In **Chapter 4** two of the eight genotypes tested in (Chapter 2) with different sources of resistance and population dynamic parameter ratios were tested for the independence of root and tuber resistance. A system was adapted to supply enough inoculum through an external source by growing a host alongside the potato plant.

Postharvest development of *M. chitwoodi* and increased quality damage was reported when infected tubers are stored (Ingham *et al.*, 2000). No detailed studies were available with regard to the effect of storage temperature and time on population dynamics of the nematodes in infected tubers. In **Chapter 5** the development of *M. chitwoodi* in infected tubers of the susceptible cv. Desiree over a time period of 240 days was studied at intervals of 60 days and at three different temperatures: 4°C for seed tubers, 8°C for ware potatoes and 12°C for potatoes for industrial processing.

There is enormous interest in the use of green manure crops in Europe. Resistant fodder radish varieties could be of major importance in managing *M. chitwoodi* population and are available for a number of years now. However, no reliable and useful information about their resistance required for a management system was available. In **Chapter 6**, the resistance of six fodder radish varieties was estimated and the possibility of a P_i independent resistance measurement was explored. The future use of green manure crops in an integrated management approach with resistant potato genotypes and winter decline to reduce population densities of *M. chitwoodi* is discussed.

Chapter 7 presents a general discussion of the preceding chapters. Some unpublished data of additional glasshouse experiments, field validation tests and simulations, based on the established parameter values, are used to discuss some possible scenarios.

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Chapter 2

A routine test for the relative susceptibility of potato genotypes with resistance to *Meloidogyne chitwoodi*

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The population dynamics of *Meloidogyne chitwoodi* on eight potato genotypes was compared to the susceptible cv. Desiree in four glasshouse experiments. The initial nematode densities consisted of log series: 2^x , with $-4 < x < 8$. Seinhorst's logistic model was fitted to the final population densities to estimate the parameters maximum multiplication rate (a), maximum population density (M) and the ratios RSa , RSM and M/a . Average RSa and RSM of the seven resistant genotypes were smaller than 0.29%. The M/a ratios on six resistant genotypes and cv. Desiree were the same, 1.3, indicating P_i independence of RS. One genotype stood out with $M/a = 8.6$, whereby $RSa < RSM$. Both RS and M/a were unaffected by pot size or experimental conditions. Screening protocols at $P_i = 24$ second-stage $J_2(\text{g dry soil})^{-1}$ in 2 or 3 kg pots were evaluated for distinctiveness between the two genotype groups. Based on the results, an optimal protocol for a routine resistance test is proposed.

Keywords: maximum multiplication rate (a), maximum population density (M), root-knot nematode, tuber infestation, tuber quality.

Introduction

Potato is the fourth major food crop in production worldwide after wheat, rice and maize (Scurrah *et al.*, 2005; FAOSTAT, 2016) and is a major export product in The Netherlands. During the last decennia, the root-knot nematode *Meloidogyne chitwoodi* has become a serious threat to potato production in the north western of the USA (Ferris *et al.*, 1993) and in European countries (Karssen, 1999). In The Netherlands *M. chitwoodi* imperils the production of seed, starch and ware potatoes as it infects potato tubers and affects tuber quality. Management by crop rotation is complicated as most arable crops are hosts (Den Nijs *et al.*, 2004) and population dynamics parameters under field conditions are largely unknown. The best strategy for the management of *M. chitwoodi* in potato rotations would be the use of crops with a low host status and resistant potato cultivars. In line with this thinking, research is now directed to the development of *M. chitwoodi* resistant potato genotypes (Norshie *et al.*, 2011; Teklu *et al.*, 2013a), resistant green manures, *e.g.*, fodder radish (Teklu *et al.*, 2014) and reliable methods to assess resistance and host status. Brown *et al.* (2006, 2009) and Janssen *et al.* (1995, 1996, 1997) reported promising wild tuber bearing *Solanum* species, *e.g.*, *Solanum bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri* and *S. hougasii*, with resistance to both *M. chitwoodi* and *M. fallax*.

Dutch breeders incorporate these genes into their marketable potato cultivars and need cheap and reliable methods to test their genotypes for resistance to *M. chitwoodi* (Draaistra, 2006). To incorporate the results of resistance testing in an advisory system for *M. chitwoodi*, they must be based on population dynamics parameters and have prescribed precision and distinctiveness with Poisson errors smaller than 10% (Been & Schomaker, 1998). For resistance, we use the definitions given by Trudgill (1991) and Seinhorst (1986), acknowledging the problems of distinguishing between zeros and small numbers in right-skewed distributions. Current methods for resistance testing, ranging from classifying tuber symptoms or reproduction rates on roots (*e.g.*, Brown *et al.*, 2006) to scoring zero nematodes in small tubes (*e.g.*, Chen & Roberts, 2003) do not qualify in either respect.

To explore the population dynamics of *M. chitwoodi* in potato, six ware and two starch potato genotypes were compared with the susceptible cv. Desiree in four glasshouse experiments, carried out from 2010 (Norshie *et al.*, 2011) until 2014 and resistance (RS) estimates based on the final population (P_f) in roots and soil were made. Seven genotypes had resistance genes from *S. bulbocastanum*, *S. fendleri* or *S. hougasii* and one had resistance genes from *S. vernei* against *Globodera pallida*. Pot sizes varied between 10, 5, 3 and 2 kg. Additionally, proportions of tubers without external and internal symptoms were estimated and, in Experiment 2, tuber infestation levels of four resistant genotypes and cv. Desiree were assessed after 240 and 300 days of storage and RS_{tuber} was estimated and compared with the $RS_{\text{roots+soil}}$ of these four genotypes. The results of the experiments are used to propose a routine test for the RS of potato genotypes to *M. chitwoodi*.

Materials and methods

All experiments were conducted in glasshouses, including the first pilot experiment by Norshie *et al.* (2011), at Wageningen University and Research. An overview of the tested genotypes sources of resistance, P_i series, pot sizes, *etc.*, is presented in Table 2.1.

Table 2.1. Overview of experimental set up used, potato genotypes tested, source of resistance (*Solanum* spp.), parent material and the breeders. Experiment 1 by Norshie *et al.* (2011). The cv. Desiree was used as a control in all experiments.

Year	Exp	Location	Pot size (kg)	Rep	Densities	Genotypes	Source of resistance	Resistant parent	Breeding company
2010	1	Wageningen	5	5	11	AR04-4096	<i>S. bulbocastanum</i>	BLBC 398.89	Agrico
						AR04-4098	<i>S. bulbocastanum</i>	BLBC 398.89	Agrico
						AR04-4107	<i>S. hougasii</i>	HOU M 94-110-2	Agrico
2011	2	Wageningen	10	4	12	AR04-4096			
						AR05-4044	<i>S. hougasii</i>	HOU M 94-110-2	Agrico
						Ka-2006/2217	<i>S. hougasii</i>	M 94-110-2	Averis
						Ka-2007/1312	<i>S. fendleri</i>	M 94-125-1	Averis
2012	3	Wageningen	10, 5, 2.4, 4, 5		12	2011M1	<i>S. fendleri</i>	M 94-144-1	HZPC
						MDG2	<i>S. vernei</i>	AM 78-3778	KWS
2014	4	Wageningen	3	5	13	AR04-4096 2011M1			

Agrico, PB 70, 8300 AB Emmeloord, The Netherlands; Averis Seeds B.V., Kweekinstituut Karna, Valtherblokken Zuid 40, 7876TC, Valthermond, The Netherlands; HZPC Holland B.V., Research & Development, PB 2, 9123 ZR Metslawier, The Netherlands; KWS POTATO B.V., Johannes Postweg 8, 8308 PB Nagele, The Netherlands.

Soil mixture

A soil mixture, free of pathogens, prepared by mixing silver sand, crushed hydro-grains and clay powder (kaolin) in a ratio of (6:1.5:1) was used in each experiment (Seinhorst *et al.*, 1995). A mixture of 1020 kg contained 725 kg silver sand, 175 kg hydro-grains, 120 kg clay powder and 1020 g of NPK (14:13:14), Osmocote, release time 3 months and 20 l of Steiner nutrient solution (EC = 2) to supplement the micro-nutrients (Steiner, 1968). The ingredients were then mixed four times, while adding 38 l of water, to ensure complete homogeneity of the growing medium. The pH of this medium was about 6.5. From each lot, a sub-sample of 1 kg was collected and kept for 24 h in an oven (105°C) to determine the moisture content.

Preparation of the pots

An overview of the dimensions of the pots used in the experiments is given in Table 2.2. The bottom of each pot had four perforations, which were covered with an Ederol filter paper number 261, 40 g m⁻² (J.H. Ritmeester B.V.) to prevent leaching of the soil. Pots were

filled with the soil mixture in four steps while gently compressing the soil to avoid soil compaction. Black plastic sheet, cut 2 to 3 cm less than the diameter of the pot, was used to cover the soil mixture on top of the pot to prevent both splashing and erosion during watering and excessive evaporation during the growing period.

Table 2.2. The dimensions of the pots used in the experiments.

Pot size (kg)	Top diameter (cm)	Base diameter (cm)	Height (cm)	Experiment
10	27	20	25	2,3
5	21	16	20	1,3
3	17	12	19	4
2	10	10	20	3

Source and preparation of inoculum

Meloidogyne chitwoodi (Mc31 or 'Smakt' population) was multiplied in 5 kg pots, on tomato (cv. Moneymaker) for ca. 3 to 5 months. At the initial population (P_i) of 4 second-stage juveniles (J2) (g dry soil)⁻¹, the multiplication rate, P_f/P_i , on cv. Moneymaker was 45. On average 9×10^5 J2(pot)⁻¹ could be retrieved. The soil was sieved through a 10 mm mesh sieve to collect the tomato roots, which were then stored in plastic bags at 4°C until further processing. One week before the tubers were planted, the roots were chopped into 1 cm pieces, placed in 20 cm diameter, 150 µm, extraction sieves. The sieves were then put on 25 cm diameter extraction dishes, which were then transferred to a mist-chamber (Seinhorst, 1988). Suspensions of hatched J2 were tapped and counted on a daily basis until the required number of inoculum was harvested. This process took 5 days at a maximum. The suspensions were stored at 4°C, aerated using aquarium pumps and eventually combined into one stock suspension, which was used to prepare all required nematode densities. Logarithmic series of nematode densities were prepared ranging from 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 J2(g dry soil)⁻¹ in pilot Experiment 1, from 0, 0.125 to 128 in Experiment 2 and 3, and from 0, 0.0625 to 128 J2(g dry soil)⁻¹ in Experiment 4. Each density had either four or five replicates.

Nematode inoculation

Suspensions of *M. chitwoodi* J2 were prepared for each density. The nematodes were inoculated into the soil of the pots first and tuber planting followed in order to mimic the natural processes in the field as far as possible, while controlling experimental variance. In order to obtain a Poisson distribution of J2 in the suspension, a plunger with a perforated circular disc was used to homogenise the suspension. We used 20, 10, 7 and 4 inoculations for the 10, 5, 3 and 2 kg pots, respectively. Needles were uniformly inserted in the pot almost at equal distance of each other and down to 2 cm from the base (Been & Schomaker, 1986). Pipettes with 3 ml of J2 suspension were placed on the needles, which were then retracted gently from the soil while releasing the suspension. This ensures an approximately random distribution of nematodes throughout the vertical profile of the soil. The holes created by the needles were closed immediately with soil.

Planting material and tuber preparation for planting

In addition to ARo4-4096, ARo4-4098 and ARo4-4107, tested by Norshie *et al.* (2011), five other genotypes, ARo5-4044, Ka-2006/2217, Ka-2007/1312, 2011M1 and MDG2, were compared with the susceptible cv. Desiree.

Seed potatoes were stored at 4°C. Two weeks before planting, tubers were placed in the dark at room temperature to initiate sprouting. Sprouts were grown up to 1 cm high and then hardened by exposing to light. A 15 mm diameter cork-borer was used to prepare uniform sized tuber pieces of 3 cm long with a single sprout, weighing 12 g each in order to reduce variation in plant growth. Immediately after inoculation of the J2, each tuber was inserted into a 6 cm deep hole in the centre of the pot made by a 18.5 mm diameter cork-borer. After sprouting, a single stem was allowed to grow.

After planting, pots were positioned randomly in the glasshouse. Redundant branches were removed. Glasshouse conditions were 18 to 20°C during the day, 16°C during the night and 16 h of day light supplemented by six 400 W, 58500 lm lamps (Hortilux Schröder). Humidity was kept at 60 to 70%. Growing periods varied from 13 to 16 weeks.

Watering

Before planting, soil moisture in the pots was raised to approximately 10% of the dry soil. After planting, moisture was maintained at 12 to 15%. Each week plant height was measured, pots were weighed and the required amount of water was added to compensate for evaporation and plant growth. Increase in plant weight was estimated from plant height. At the same time, pots were rotated within the glasshouse to avoid positional effect. In between weighing, when water use was presumed to be high, five randomly selected pots were weighed to assess water loss and an equal amount of water was added to all the pots.

Storage conditions of potato tubers

Harvested potato tubers from Experiment 2, originating from the genotypes ARo4-4096, ARo5-4044, Ka-2006/2217, Ka-2007/1312 and the susceptible cv. Desiree, were stored for 240 days (1st and 2nd replications) and 300 days (3rd and 4th replications) to estimate the infestation levels of *M. chitwoodi* in potato tubers. The tubers were divided into two batches because of limited space in the spray-mist chamber. Throughout the storage period, a temperature of 7°C and humidity of 99% was maintained with adequate ventilation. The average storage period and conditions were in accordance with common practice for ware potatoes.

Nematode extraction

The P_f was estimated by extracting and counting the nematodes from the roots, the soil and the peel from tubers (Experiment 2).

P_f from potato roots and soil

Whole root systems were used to minimise variation. After harvest, potato roots were collected using sieves with dimensions of 49 cm length \times 32 cm width and 8 cm height and mesh size of 6 \times 6 mm, gently rinsed with water to remove any soil attached and fresh root weights were determined. Then the roots were cut into 1 cm pieces and placed in 9 cm diameter, 150 μ m extraction sieves. The sieves were then put on top of 12 cm extraction dishes and were kept in a spray-mist chamber (Seinhorst, 1988) at 20°C for 4 weeks. Hatched J2 were collected at 7-day intervals. A 500 g subsample of the well-mixed soil was elutriated using the Seinhorst elutriator to estimate the number of nematodes in the soil (Seinhorst 1962, 1988; Teklu *et al.*, 2014).

P_f in potato tubers

In Experiment 2 the $P_{f_{\text{tuber}}}$ was estimated for the resistant genotypes ARo4-4096, ARo5-4044, Ka-2006/2217, Ka-2007/1312 and cv. Desiree. After 240-300 days of storage, tubers were exposed to 20°C for 2 weeks to harden their skin. All tubers larger than 25 mm diameter in the middle of the longest side were peeled 5 mm deep (Viaene *et al.*, 2007). The peel was then cut into 1 cm² pieces and placed on 20 cm diameter, 425 μ m extraction sieves. The sieves were then put on 25 cm diameter extraction dishes and kept in a spray-mist chamber for 7 weeks, while hatched J2 were collected and counted every 7 days. The chosen mesh size for tuber peel was larger than for roots to avoid clogging of the sieves by starch. The logistic hatching curve of the susceptible cv. Desiree was used as a reference for the genotypes to monitor the hatching process and to determine the time to stop the test.

In all experiments, the average percentage of tubers without external or internal symptoms, F_0 , was estimated.

Nematode counts

To estimate P_f values, and hence the population dynamics parameters, accurately, at least 200 nematodes from all fractions should be counted, assuming a Poisson distribution of the nematodes in well-mixed suspensions, to keep coefficients of variation below 10%. This prerequisite was not always realised, mostly because of small nematode numbers in highly resistant genotypes.

Data analysis and model fitting

Population dynamics (P_f from roots and soil)

Data analysis, model fitting and parameter estimation were done using scripts written in Tinn-R editor version 4.0.2.1 and run in R-version 3.2.2. The procedure was as follows:

- i) After log transformation of the final population densities (P_f), the means per set of replicates were estimated and back transformed. Zeros were replaced by half the minimum of the non-zero values within a set of replicates. They were not considered to be 'true' zeros but samples from right-skewed distributions that contain small numbers, close to zero (Teklu *et al.*, 2014)
- ii) To estimate the population dynamic parameters, the maximum multiplication rate (a) and the maximum population density (M), the logistic model (Equation 1) (Seinhorst, 1966, 1967, 1970) was fitted to the data, using Ordinary Least Squares.

$$P_f = M \times P_i / (P_i + M / a) \quad (1)$$

Their standard errors and covariance of the parameters were obtained from the inverse of the Hessian matrix (Venables & Smith, 2012).

- iii) To evaluate differences in identical parameters of the tested genotypes, differences of the log transformed values were calculated and compared with Least Significant Differences (LSD), estimated from standard errors (Equations 2 and 3) and the critical values, with $P = 0.05$ (Dalgaard, 2002).

$$SE^2(X) = \log(CV_x^2 + 1) \quad (2)$$

$$SE_{X1+X2}^2 = SE_{X1}^2 + SE_{X2}^2 \quad (3)$$

X is $\log(a)$ or $\log(M)$; x is a or M while CV_x is the coefficient of variation of x . X_1 and X_2 are two identical, log transformed, parameters.

- iv) As the shape of the $P_i \sim P_f$ curves is decided by the ratio of the parameters M and a (Seinhorst *et al.*, 1995), M/a ratios and their expected values were first calculated per genotype, per experiment. The coefficients of variation of the M/a ratios were estimated with the Taylor series (Mood *et al.*, 1974), resulting in Equation 4.

$$CV_{A/B}^2 \approx CV_A^2 + CV_B^2 - 2 \cdot \text{cov}_{A,B} / \mu_A \mu_B \quad (4)$$

In Equation 4, A is nominator and B denominator in a ratio of A and B , whilst μ_A and μ_B are the expected values of A and B . The differences between the log transformed M/a ratios were compared with LSD in the same manner as the differences between the log transformed parameters (Equation 3). Second, per group of genotypes with the same M/a ratio, averages and their variances were estimated, after log transformation. Equation 2 was used to estimate CVs from the variances of $\log(M/a)$: only now $X = \log(M/a)$, while $x = M/a$.

- v) For each genotype percentages of Relative Susceptibility, RSa and RSM , based on the P_f in the roots and soil, were estimated.

$$RSa = 100 \times a_{\text{res}} / a_{\text{sus}} \quad (5)$$

$$RSM = 100 \times M_{\text{res}} / M_{\text{sus}} \quad (6)$$

The subscripts *res* and *sus* refer to the resistant test genotype and the susceptible reference cv. Desiree, respectively.

The differences between RS estimates between genotypes were evaluated in the same way as the M/a ratios. The exception was that the third term of Equation 4, the covariance, was assumed negligible.

- vi) For a given genotype, RS_a equals RSM only when its M/a ratio equals that of the susceptible standard, cv. Desiree, (Seinhorst *et al.*, 1995). Therefore, the impact of the estimated M/a ratios on the shape of $P_i \sim P_f$ curves (parallelism or lack thereof) and the choice of a suitable test- P_i value were explored by estimating the P_i values where 95% of the P_f -limit, M , was obtained, using Equation 1.
- vii) Using the estimates for a and M , average nematode counts per P_i value in 0.2, 0.5, 1, 2 and 3 kg pot sizes or sample sizes were estimated. The Poisson distribution was used to estimate the 0.001 and 0.999 quantiles of these counts. From these calculations, the necessary pot sizes and subsamples were estimated to keep coefficients of variation smaller than 10% by counting at least 200 nematodes in the total P_f .

Population dynamics (P_f from potato tubers)

By definition, the nematodes obtained from the tubers are also part of the final population density. However, these nematodes do not remain in the soil, but are transported with the harvested tubers and might cause new infestations. Therefore, the P_f in the potato tubers of Experiment 2 was modelled separately and their percentage of the total P_f was calculated. Nematodes extracted from potato tubers of four replicates that were 240 and 300 days in storage were combined as numbers of hatched J2 from the two batches were the same. The hatching process in the mist chamber was followed weekly and a logistic model (Equation 7) was used to estimate the hatching parameters C , B and A , in the cases where enough nematodes were collected to fit the model.

$$F(t) = \frac{C}{(1 + \exp(-B \times (t - A)))} \quad (7)$$

Where

C	maximum cumulative hatched J2(g peel) ⁻¹ , expressed J2(g dry soil) ⁻¹
B	relative rate of hatching (dimensionless)
A	time when $0.5 \times C$ is reached in (days)
t	hatching time (days)

To compare P_f from roots and soil with P_f from the tubers, $P_{f_{\text{tuber}}}$, expressed as J2(g dry soil)⁻¹ was estimated from the parameter C , the fresh peel weight per pot and g soil per pot. The RS_{tuber} was estimated by calculating $P_{f_{\text{total}}}$ (P_f from roots, soil and tubers) and estimating the percentage $P_{f_{\text{tuber}}}$ of the four tested genotypes.

Results

Population dynamics in roots and soil

Model fit and parameter estimates

The parameter estimates (specified per experiment and per genotype), coefficients of variation (CV) and relevant regression output, are summarised in Table 2.3. Equation 1 fitted well in 17 of the 21 combinations. In Figures 2.1 and 2.2, data and fitted models are displayed for the genotypes tested in Experiment 2 and Experiment 3. For four combinations, where $R^2 < 0.2$ or not available (NA), the parameter a could either not be estimated, or not estimated with sufficient accuracy, mostly because the nematode counts were too small or lack of regression in the data. This means that the parameter M was already reached at the smallest P_i values (Experiment 1 and Experiment 4). When regression analysis was not possible, M and CV_M were estimated directly from the P_r .

Table 2.3. Parameter estimates from the non-linear regression analysis of Equation 1; $P_r = M \times P_i / (P_i + M/a)$ Where: a = maximum multiplication rate; M = maximum population density in J2(g dry soil)⁻¹ of *Meloidogyne chitwoodi*; CV_a , CV_M and $CV_{M/a}$ = coefficients of variation of a , M and M/a ratio, estimated from the Hessian Matrix, RS_a and RSM (%) = Relative Susceptibility, df = degrees of freedom; R^2 = the proportion of variance explained by Equation 1.

Genotype / cv	Exp	Pot size (kg)	a	M	M/a	CV_a	CV_M	$CV_{M/a}$	RS_a (%)	RSM (%)	df	R^2
ARo4-4107	1	5	0.55	0.16	0.30	1.22	0.19	1.32	1.70	0.20	8	-0.01*
ARo4-4098	1	5	0.47	0.12	0.26	1.97	0.26	2.12	1.45	0.15	8	-0.08*
ARo4-4096	1	5	0.27	0.18	0.67	1.43	0.41	1.65	0.83	0.22	8	-0.02*
Desiree	1	5	32.16	80.09	2.49	0.30	0.16	0.39	100.00	100.00	8	0.73
ARo4-4096	2	10	0.02	0.12	6.89	0.65	0.70	1.20	0.04	0.21	8	0.55
ARo5-4044	2	10	0.12	0.13	1.14	0.49	0.32	0.66	0.26	0.23	9	0.53
Ka-2006/2217	2	10	0.04	0.08	2.12	0.33	0.27	0.51	0.08	0.14	9	0.74
Ka-2007/1312	2	10	0.14	0.36	2.66	0.15	0.13	0.23	0.31	0.64	9	0.95
Desiree	2	10	44.35	56.67	1.28	0.25	0.24	0.42	100.00	100.00	6	0.84
2011M1	3	10	0.09	0.76	8.63	0.20	0.25	0.36	0.14	1.45	9	0.92
MDG2	3	10	42.21	44.01	1.04	0.17	0.11	0.24	69.54	84.56	9	0.90
Desiree	3	10	60.69	52.05	0.86	0.29	0.16	0.39	100.00	100.00	9	0.74
2011M1	3	5	0.25	1.96	7.78	0.17	0.22	0.31	0.57	4.01	9	0.94
MDG2	3	5	27.39	65.70	2.40	0.14	0.11	0.21	62.38	134.67	9	0.95
Desiree	3	5	43.90	48.79	1.11	0.13	0.08	0.17	100.00	100.00	9	0.94
2011M1	3	2	0.58	3.70	6.36	0.18	0.21	0.31	0.49	2.02	9	0.94
MDG2	3	2	102.08	166.54	1.63	0.18	0.13	0.25	85.56	90.68	9	0.91
Desiree	3	2	119.31	183.66	1.54	0.15	0.11	0.21	100.00	100.00	9	0.93
ARo4-4096	4	3	NA	0.02	NA	NA	0.29	NA	NA	0.02	10	NA
2011M1	4	3	0.05	0.63	13.32	0.25	0.40	0.53	0.07	0.96	10	0.90
Desiree	4	3	69.20	65.89	0.95	0.10	0.07	0.14	100.00	100.00	10	0.97

Most notable are the results in the 2 kg cylinder pots (Experiment 3) where the population dynamics parameters were increased almost threefold for all genotypes, although M/a ratios were not influenced by pot size.

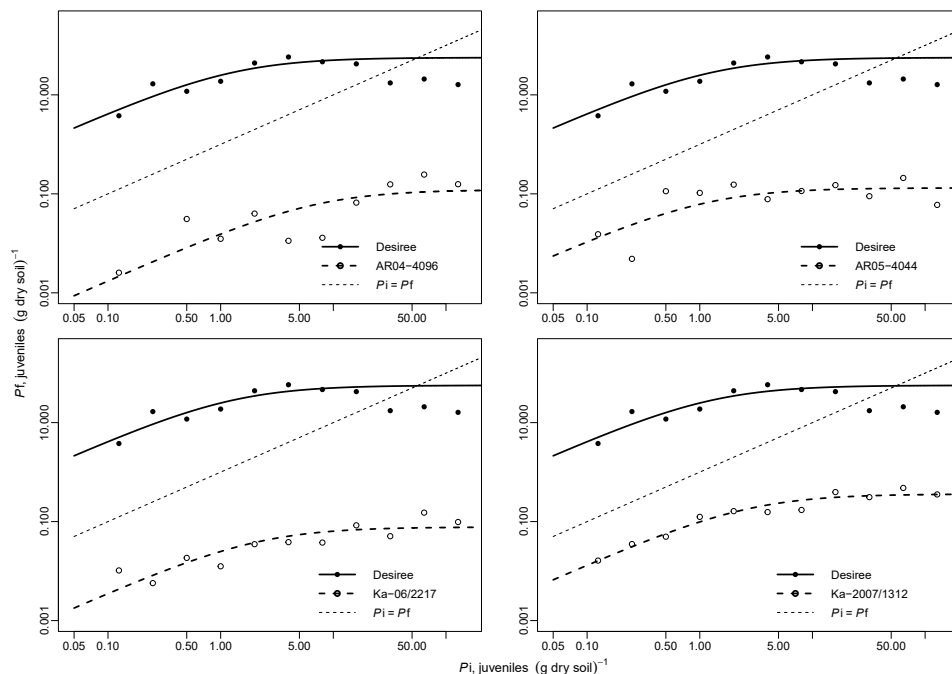


Figure 2.1. The relation between initial (P_i) and final population density (P_f) of *Meloidogyne chitwoodi* second-stage juveniles, J_2 (g dry soil) $^{-1}$ on a log scale in Experiment 2. Fitted lines according to Equation. (1): $P_f = M \times P_i / (P_i + M/a)$ (Seinhorst, 1966). The parameters a and M are the maximum multiplication rate and the maximum population density, respectively. Comparisons were made between cv. Desiree (solid line) and the genotypes AR04-4096, AR05-4044, Ka-06/2217 and Ka-07/1312 (dashed-lines). The diagonal fine broken lines represent the equilibrium line ($P_i = P_f$).

On cv. Desiree, in all experiments and in all pot sizes except the 2 kg pots, the parameters a and M were remarkably constant, averaging 48.3 and 59.7, respectively (Table 2.3). The Coefficient of variation, CV_a and CV_M between experiments were estimated to be 0.14 and 0.09. However, in Experiment 1 and Experiment 2, P_f on cv. Desiree decreased at $P_i \geq 32$ J_2 (g dry soil) $^{-1}$. This did not happen on the resistant genotypes.

As expected, the genotype MDG2 with resistance genes only to *G. pallida*, proved to be susceptible to *M. chitwoodi* (Figure 2.2), with averages for a and M of 33.9 and 53.7 J_2 (g dry soil) $^{-1}$, respectively. Between experiments, CV_a and CV_M were both 0.2 and higher for cv. Desiree.

In the 10, 5 and 3 kg pots, no differences in parameter values of the resistant genotypes was detected and average values of a and M of 0.09 and 0.12 were observed, with one exception: parameter values of M on 2011M1 were higher in all experiments and an average of about 1 J_2 (g dry soil) $^{-1}$ was found.

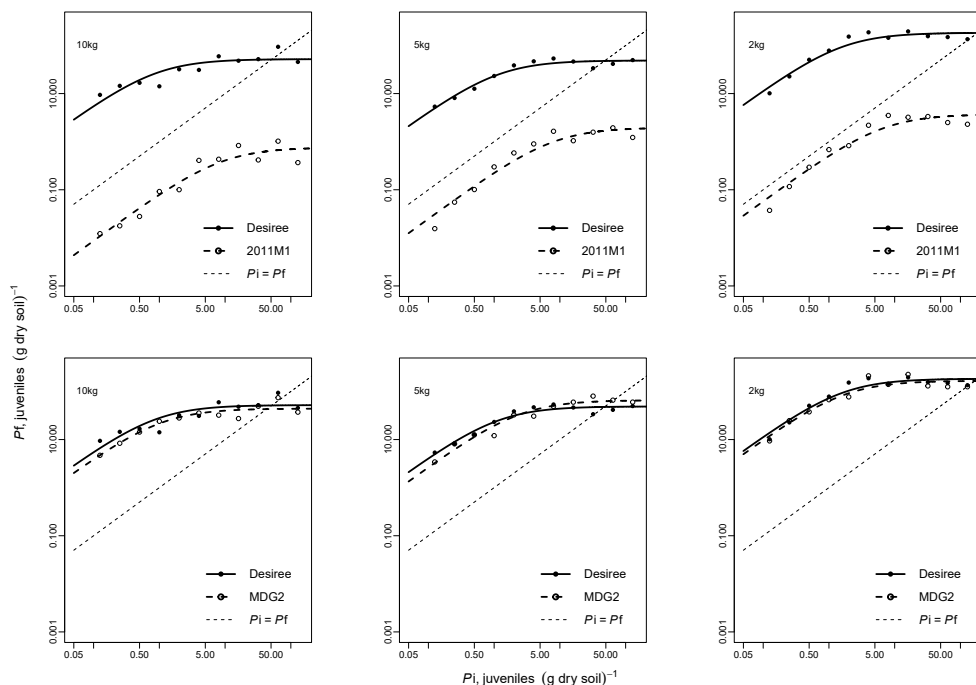


Figure 2.2. The relation between initial (P_i) and final population density (P_f) of *Meloidogyne chitwoodi* second-stage juveniles, $J_2(\text{g dry soil})^{-1}$ on a log scale in Experiment 3. Fitted lines according to Equation 1: $P_f = M \times P_i / (P_i + M/a)$ (Seinhorst, 1966). The parameters a and M are the maximum multiplication rate and the maximum population density, respectively. Comparisons were made between cv. Desiree (solid line) and the genotypes 2011M1 and MDG2 (dashed-lines) in 10, 5 and 2 kg pots. The diagonal fine broken lines represents the equilibrium line ($P_i = P_f$).

Ratios of maximum population density to maximum multiplication rate (M/a)

The M/a ratio was not influenced by pot size and on 8 out of 9 genotypes, including cv. Desiree, the same average was found: 1.3 ($CV_{M/a} = 0.21$). An exception was 2011M1 with an average M/a ratio of 8.6 ($CV_{M/a} = 0.16$).

Nematode counts

The 0, 2.5, 50, 97.5 and 100% quantiles of nematode counts for all genotypes and cv. Desiree were calculated. All experiments combined, the nematode counts to estimate the P_f on cv. Desiree varied between 96 and 4970 nematodes per pot and 97.5% of the counts were higher than the required 200 nematodes. By comparison, the nematode numbers counted on 2011M1 varied between 0 and 424, with a median of 28 nematodes. Only 10% of the nematode counts on 2011M1 was more than 200. For Ka-2006/2217, all counts were lower than 40 nematodes.

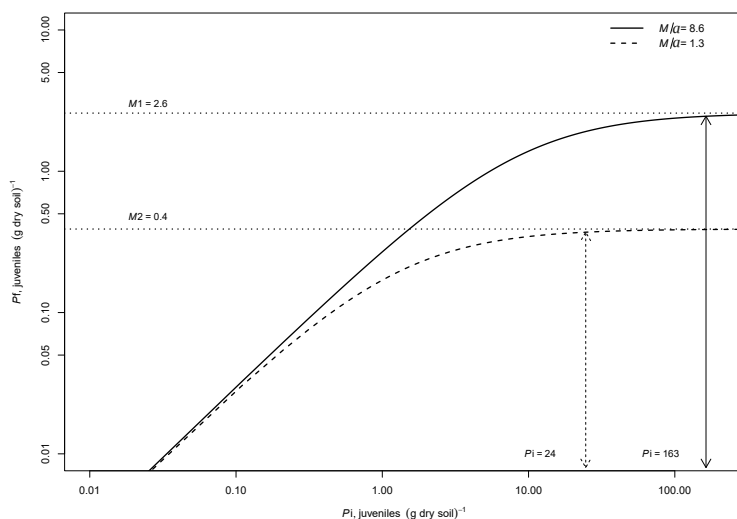


Figure 2.3. where P_i is plotted against P_f of *Meloidogyne chitwoodi* for both the resistant genotypes with M/a ratios of 1.3 and 8.6, shows that the P_i at which P_f comes close to a certain proportion of M , which is related to M/a . From Equation 1 we can derive that $P_i = p \times M$ when $P_f = (p / (1 - p)) \times M / a$. The variable p is the proportion of M we choose for our test P_i in a routine test. For convenience, we indicate the P_i value, where the corresponding P_f equals $p \times M$, as P_{ipM} . As a consequence, $P_{10.95 \times M}$ for cv. Desiree and all genotypes except 2011M1, was rounded down, $1.3 \times 19 = 24$ J2(g dry soil)⁻¹. For genotype 2011M1, $P_{10.95 \times M}$ was estimated to be $8.6 \times 19 = 163$ J2(g dry soil)⁻¹.

Based on the population dynamic results in the 10, 5 and 3 kg pots, presented in Table 2.3, 0.001 and 0.999 quantiles from the Poisson distribution, with lambda = nematode counts, were calculated to investigate how (in terms of P_i values or ranges, pot sizes and (sub- sample sizes) the requirement in the introduction, $CV_{\text{Poisson}} < 10\%$ or nematode counts (= lambda) > 200, can be satisfied. The results of these simulations, and the two $P_{10.95 \times M}$ values, are summarised in Figure 2.4. Only the 2 kg and 3 kg pots meet the requirements of 200 nematodes in the P_f per pot, when the whole pot content is investigated. For genotypes, such as 2011M1 subsamples with higher M values of 500 g are sufficient. If we choose the 2 kg cylinder pots, subsamples could be reduced to 500 g because of the higher estimates for a and M (Figure 2.5).

Relative susceptibility (roots and soil)

In all experiments and in all pot sizes the average R_{Sa} of all genotypes, MDG2 excluded for lack of resistance genes against *M. chitwoodi*, was 0.29% with a CV of 0.21. The average R_{SM} of all genotypes, except MDG2 and 2011M1 (the latter was excluded because of the higher M/a ratio) was 0.23% with a CV of 0.09, due to smaller counting errors (derived from Table 2.3). The average R_{Sa} equalled the average of R_{SM} ($P = 0.53$). Genotype MDG2 had higher R_{Sa} and R_{SM} estimates (> 60%) in all pot experiments.

F0, percentage tubers without internal or external symptoms

The percentage Fo produced by cv. Desiree and MDG2 was 7.3%, while 2011M1 and the remainder of the resistant genotypes (ARo4-4096, ARo4-4098, ARo4-4107, ARo5-4044, Ka-2006/2217 and Ka-2007/1312) produced, on average, 53% and 91% nematode free tubers, respectively.

P_f in tubers after 240-300 days of storage (Experiment 2)

The logistic curve (Equation 7) fitted well to the number of hatched J2 of *M. chitwoodi* from tubers of cv. Desiree stored for 240-300 days, but not to J2 from tubers of the four tested resistant genotypes, due to their small numbers. The estimate of the parameter C was considered the $P_{f_{\text{tuber}}}$ of cv. Desiree. As no regression was found between P_i and $P_{f_{\text{tuber}}}$, all data were pooled and the average $P_{f_{\text{tuber}}}$ on cv. Desiree was estimated from the $P_{f_{\text{tuber}}}(\text{pot})^{-1}$ and soil pot^{-1} to be $0.35 \text{ J2}(\text{g dry soil})^{-1}$, 1.9% of the total P_f (the P_f from roots, soil and tubers). The $P_{f_{\text{tuber}}}$ on the resistant genotypes, estimated from the cumulative hatch, was $0.002 \text{ J2}(\text{g dry soil})^{-1}$, 2.1% of the total P_p , the same percentage as on cv. Desiree tubers. This implies that the $RS_{\text{roots+soil}}$ estimates of the four resistant genotypes tested in Experiment 2 equal RS_{tuber} estimates.

Discussion

Estimates of Relative Susceptibility

In total eight genotypes were tested for their resistance, some of them replicated. Apart from MDG2, without resistance genes against *M. chitwoodi* and 2011M1 (different *M/a* ratio) the estimates for RSa and RSM of all tested genotypes were approximately the same. Relative Susceptibility (RSM) and *M/a* ratio of genotype 2011M1 was always significantly higher than those of other resistant genotypes. Both genotypes 2011M1 and Ka-2007/1312 share the same source of resistance *S. fendleri* (seedling 93-114-5) but differ in their genetic background, due to backcrossing with Y68-4-103 or cv. Bildtstar, respectively. This resulted in two different resistant parents: M94-144-1 to 2011M1 and M94-125-1 to Ka-2007/1312. Most likely, the history of backcrossing influenced the expression of the resistance gene. The standard breeder's resistance tests (counting root-knots on potato roots grown in small tubes, a procedure similar to that of Wesemael & Moens (2012), failed to differentiate between the two parents and to detect 2011M1 as an aberrant genotype. Therefore, breeders want more accurate tests for both root and tuber resistance and tolerance preferably, one test for both.

In our experiments, both RS estimates were independent of glasshouse conditions, as was the case for the RS of potato cultivars resistant to potato cyst nematodes (PCN) (Seinhorst *et al.*, 1995). The RS of PCN was the same for field and glasshouse conditions (Phillips, 1984; Molendijk, pers. comm.). Therefore, most probably, the estimates for *M. chitwoodi* will also

apply under field conditions. To check this assumption and to predict the actual P_f on the resistant genotypes in the field, experiments are required to estimate a and M of at least the susceptible reference cv. Desiree and the maximum P_f and M of some resistant genotypes under field conditions. Estimating the parameter a under field conditions with sufficient accuracy would be impossible, due to the small nematode numbers and the increased sampling variance.

Requirements to estimate RS

Based on the prerequisites in the introduction and population dynamics data in the results, we can now specify the requirements for RS tests of potato genotypes in more detail:

- i) $RSa = RSM$, implying that the $P_i \sim P_f$ lines of resistant genotypes and the susceptible standard run parallel on a log scale and that M/a ratios of all genotypes are the same;
- ii) P_i must be chosen so that genotypes with deviating M/a ratios can be detected;
- iii) P_f reduction at the test P_i due to growth reduction should be negligible;
- iv) Pot size or sub sample sizes should be chosen so that $CV_{\text{Poisson}} < 10\%$ or nematode counts in the P_f (λ) = 200;
- v) Root resistance should be a measure for tuber resistance; and
- vi) The percentage of tubers without internal or external symptoms should be estimated as a measure of tolerance for export potatoes.

Equality of RS estimates ($RSa = RSM$)

The M/a ratio of the seven out of eight tested genotypes and cv. Desiree was the same, 1.3. Only genotype 2011M1 differed from cv. Desiree and the other genotypes in all experiments, because of the higher M parameter relative to a . For breeders and farmers, it is important that these genotypes be detected early in a routine resistance tests as they may build up higher population densities and result in lower tuber quality. For comparison, the percentage of 'nematode-free' tubers (F_0) of 2011M1 with $M/a = 8.6$ was 53, against 91 of the resistant genotypes with $M/a = 1.3$. Therefore, the deviant M/a ratio of 2011M1 was associated with higher quality loss in tubers. For this relation to be predictable, we have to find the biological mechanism behind M/a ratios and tuber quality. A time series of nematode population in relation to potato growth and development could throw light on this phenomenon.

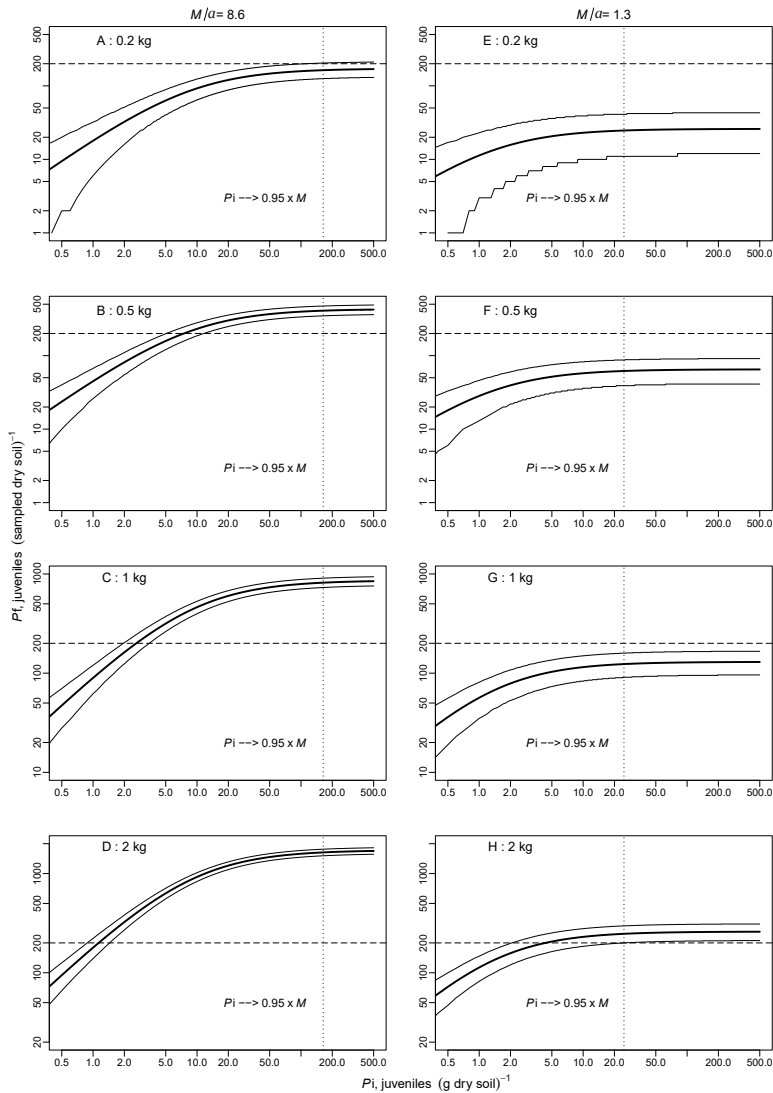


Figure 2.4. The relations between initial (P_i) and final (P_f) population density expressed as *Meloidogyne chitwoodi* second-stage juveniles, $J_2(\text{g dry soil})^{-1}$ and $J_2(\text{sample})^{-1}$, respectively, based on multiplication rates in all pot sizes, except the 2 kg cylinder pots. Sample size varied between 0.2 and 2 kg. A-D: refer to the genotype where $M/a = 8.6$. E-H: refer to genotypes where $M/a = 1.3$. The parameters a and M are the maximum multiplication rate and the maximum population density, respectively. The bold solid lines represent the 50% quantile and the thin lines are 99.9% and 0.1% quantiles. The horizontal dashed line indicates the minimum count of 200 nematode required to obtain $CV < 0.1$ and the vertical broken lines indicates the P_i where $P_f = 0.95 \times M$ is reached.

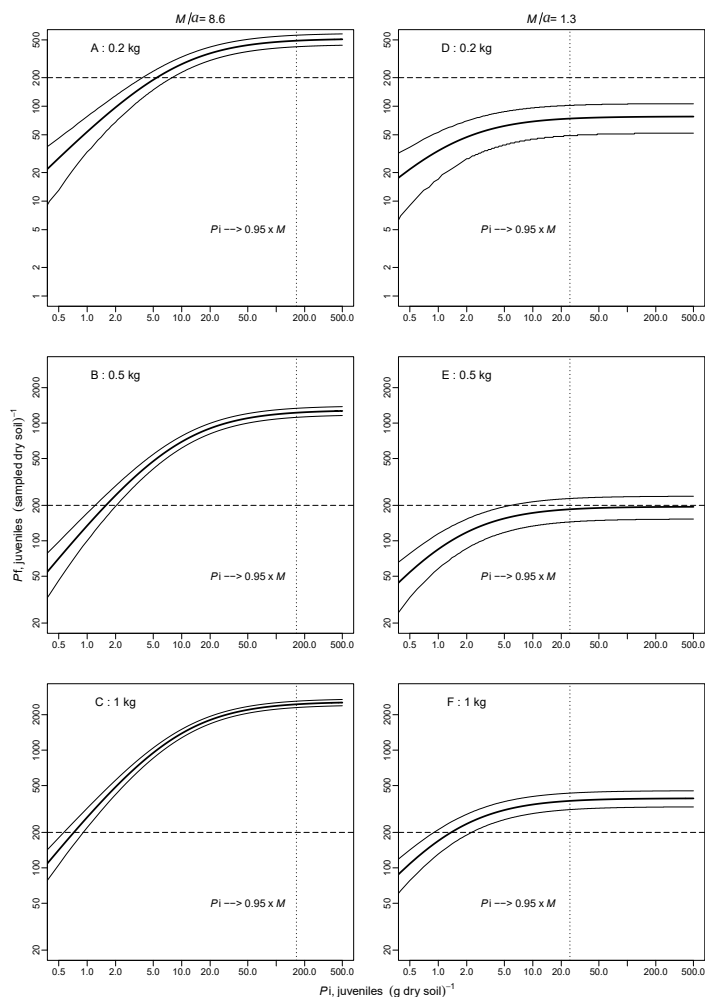


Figure 2.5. The relation between initial (P_i) and final (P_f) population density measured as *Meloidogyne chitwoodi* second-stage juveniles, $J_2(\text{g dry soil})^{-1}$ and $J_2(\text{sample})^{-1}$ based on multiplications rates in the 2 kg cylinder pots. Sample size varied between 0.2 and 1 kg. A-C: refer to the genotype where $M/a = 8.6$. D-F: refer to genotypes where $M/a = 1.3$. The parameters a and M are the maximum multiplication rate and the maximum population density, respectively. The bold solid lines represent the 50% quantile and the thin lines are 99.9% and 0.1% quantiles. The horizontal dashed line indicates the minimum count of 200 nematode required to obtain $CV < 0.1$ and the vertical broken lines indicate the P_i where $P_f = 0.95 \times M$.

Meloidogyne chitwoodi is not the only plant-parasitic nematode with aberrant M/a ratios on resistant potato cultivars. Seinhorst *et al.* (1995) found two out of eleven ($\approx 18\%$) PCN resistant potatoes with deviating M/a ratios. Therefore, he suggested that PCN resistant cultivars should be tested at two, relative small, P_i values. Arbitrarily, only one test P_i of 5 eggs and $J_2(\text{g dry soil})^{-1}$ in 2 kg pots was chosen for routine testing in The Netherlands in 2000 and subsequently in the EPPO region in 2006 (Anonymous, 2006). Since the introduction of highly resistant potato cultivars against PCN in The Netherlands in the

1990s, farmers and their advisors often observe higher nematodes densities on some of the PCN resistant cultivars than would be expected from their RS estimate. This phenomenon is often attributed to an increase in virulence of the PCN population or to errors in the RS tests of PCN resistant cultivars. However, another reason could be a deviance in M/a ratio caused by a relative higher M value.

Detection of genotypes with deviating M/a ratios

As shown in Figure 2.3, which presents the relation between P_i and P_f of genotypes with M/a values of 1.3 and 8.6, we observed that at a high P_i value, like $163 \text{ J2(g dry soil)}^{-1}$, where $P_{10.95 \times M}$ is reached for genotypes with $M/a = 8.6$, there was considerable difference between the M estimates of the test genotypes. However, routine test for resistance at these densities would require impractically large amounts of inoculum. Moreover, at high P_i test values P_f estimates may be influenced by differences in the tolerance parameter m (relative minimum plant weight), especially for cv. Desiree at $P_i \geq 32 \text{ J2(g dry soil)}^{-1}$, resulting in decreased P_f values of the susceptible standard and overestimation of RS. Should we choose $P_i = 24 \text{ J2(g dry soil)}^{-1}$ for testing (the P_i where 95% of M is reached for cv. Desiree and all other tested genotypes, except 200M1), we can still discriminate between the genotypes with $M/a = 8.6$ and $M/a = 1.3$. To estimate the necessary CV of both $\log(P_f)$ estimates and degrees of freedom, while $P = 0.05$, we perform a two sided t-test backwards, starting with the expected difference between the $\log(P_f)$ estimates on the test genotype and the resistant reference, over LSD to the CV of the P_f estimated from SED of the log transformed P_f values (Equation 2). Although there are more options, as LSD is determined by the degrees of freedom, SED and the 0.975 quantile of the Student Distribution, a CV of 16% and 8 degrees of freedom, amounting to five repetitions per P_i value, per genotype, would suffice. If this CV level is impossible to realise in routine laboratories, then the number of repetitions will have to be increased accordingly.

Final population (P_f) not influenced by growth reduction

In all four experiments, P_f of the resistant genotypes did not decrease at high P_i values due to growth reduction by *M. chitwoodi*. This was not always true for cv. Desiree, but P_f only decreased at the three highest P_i values, from $32 \text{ J2(g dry soil)}^{-1}$ onwards. This can be considered fortunate, because for *M. chitwoodi* we now can choose a relative high P_i value to estimate the RS. Consequently, we will obtain higher nematode counts and fewer zero nematode counts in the P_f and, subsequently, less variation in the RS estimates. By way of comparison, for PCN a smaller P_i of 5 eggs and $\text{J2(g dry soil)}^{-1}$ was necessary to avoid biases because of differences in tolerance (parameter m) between cultivars.

Pot size and nematode counts

Pot size did not affect the M/a ratios. Therefore, the pot size used only has to be sufficiently large to produce enough nematodes to count at least 200 nematodes in the P_f if the P_i value is $24 \text{ J2(g dry soil)}^{-1}$. Based on the estimates for a and M in the 10, 5 and 3 kg pots, pot sizes or subsamples of at least 2 kg are needed to meet the counting requirements (Figure 2.4).

However, because of the higher values of the population dynamics parameters these pots produce, the 2 kg cylinder pots may offer advantages. If these parameter estimates prove to be consistent, pot or subsample sizes can be reduced to 0.5 kg. Presently, some elutriators, *e.g.*, the rebuilt Seinhorst elutriator (Teklu *et al.*, 2013b), are able to produce a relatively clear suspension when 500 to 800 g of the soil mix is processed, which means that the required 500 g of soil can be handled as one subsample. However, these cylindrical pots were only tested in one experiment and additional tests are needed.

The variation due to the Poisson or more clustered distributions of nematodes in roots and soil is easily underestimated, especially when poor hosts or highly resistant plants are evaluated. Finding zero nematodes or absence of eggs masses in small tubes (Chen & Roberts, 2003; Wesemael & Moens, 2012) is not an indication of 100% resistance but merely a matter of chance as the probability of zeros increases steeply with decrease of sample size from right-skewed distributions (Mood *et al.*, 1974; Been & Schomaker, 1998). To illustrate this: a needle in a haystack will most probably never be found, no matter how long one searches for it, but investigating one straw is pointless. Therefore, basically, all resistance will be partial, as we cannot prove it to be otherwise.

Equality of root and tuber resistance ($RS_{\text{root + soil}} = RS_{\text{tubers}}$)

Brown *et al.* (2009) distinguished two linked genetic factors that control resistance to *M. chitwoodi* race 1 in the roots and tubers of potato plants independently. Since then, there is doubt whether or not root resistance equals tuber resistance. It is difficult to relate the results of glasshouse and field tests of Brown *et al.* (2009) to our data, as their methods deviate greatly from ours in at least two respects. First, Brown *et al.* (2009) did not estimate population dynamic parameters but distinguished three classes of reproduction rates. Second, there is a vast difference between their definition of “not-culled” tubers (< 6 brown spots) and Fo, the percentage of symptom-free potato tubers, although both are measures of tolerance, not resistance. Nevertheless, the RS_{tuber} of four resistant genotypes, estimated after 240 and 300 days of storage, equalled their $RS_{\text{root + soil}}$ but it is unclear if and how these data are related to tolerance.

The proposed test

In the proposed test, below, we try to remedy the shortcomings of current resistance tests, based on the results in this paper.

Foremost, materials and methods are crucial. For instance, nematodes should be present and distributed randomly in the soil at the time of planting, while tubers should not have root systems at their first encounter with nematodes, as this affects the tolerance parameter m (relative minimum plant weight) of plants and population dynamic parameters of the nematodes (Seinhorst, 1995). We propose a single initial population density (P_i) of 24 J2(g dry soil)⁻¹. To keep $CV_{\text{count}} < 10\%$, equivalent to counting 200 nematodes if variation due to personal errors is ruled out, the desired pot and sub-sample size is 2 kg, but sub-samples

can be reduced to 500 g when the higher parameter values (a and M) in the cylindrical 2 kg pots (Experiment 3) can be confirmed. To keep coefficients of variations of the difference between log transformed P_f values of the test cultivar and a resistant reference cultivar smaller than 16%, there should be at least five replicates per cultivar. We propose cv. Desiree as the susceptible reference cultivar, as many data of this cultivar are already available and we are fairly confident that its M/a ratio equals 1.3 and the tolerance at $P_i \leq 24 \text{ J}_2(\text{g dry soil})^{-1}$ is sufficient. For the same reasons we propose ARo4-4096 as the resistant reference. As long as we do not understand the biological background of the relation between the M/a ratio and tuber infestation, and F_o , the percentage of symptom-free tubers, both F_o and RS_{tuber} should be estimated. We propose the 'Smakt' or Mc31 population as international reference: it was used in the DREAM (EU QLRT-1999-1462) project for development of resistant potatoes and fodder radish and since then, as the test population in The Netherlands, Belgium and Germany. It is important that per country, especially potato importing countries, at least two virulent local population be added to the test, to enable evaluating local population dynamics. We recommend testing all genotypes twice. The question of the optimum harvest time should be resolved by studying P_f time series in relation to plant growth, so that any relevant differences between early and late varieties will not interfere with the RS estimate.

When a difference is found in the test between P_f estimates of the resistant test genotype and the resistant reference genotype there are two possibilities: either both RSa and RSM are increased or – like 2011M1 – only RSM , but not RSa , is increased. The fate of these genotypes then depends on their intended end use and tuber tolerance. It is up to the breeders to decide if it is worthwhile to submit these genotypes for further tests.

Finally, the methodology of the glasshouse test and the extraction of nematodes from the roots and soil, including extraction efficiency and variation, should be uniform between laboratories and described in detail in standard operating procedures (SOPs) as they will influence the reliability of the RS estimate greatly. Obviously, laboratory personnel must be skilled, well trained and be capable of producing stable results.

Final considerations

There are some knowledge gaps, important to resistance testing. The two most important ones are the duration of the resistance test and possible differences in virulence of *M. chitwoodi* population.

Growing period

The RS may also be influenced by the growing period of the resistant and susceptible potatoes. In our experiments, the growing period varied between 13 and 16 weeks, depending on the senescence of the plants (early and late varieties). Time series of P_f values on the resistant genotypes and cv. Desiree will provide better information about the optimum harvest time. Crucial is the time where P_f reaches its maximum, dependent on root growth, and the rate of decrease, thereafter.

Virulence of *Meloidogyne chitwoodi* population

All tests were carried out using the Mc31 or 'Smakt' population of *M. chitwoodi*, which is used in all test programmes in The Netherlands (e.g., for fodder radish testing). Up to now, no other *M. chitwoodi* populations are characterised in terms of population dynamics parameters, a and M , on a selection of susceptible and resistant potato genotypes. Consequently, a suitable number of populations have to be tested for their virulence and the most virulent population must be selected for both the screening for new promising genotypes and for the final routine resistance test in a resistance-testing programme. Presently, in The Netherlands, it is not clear whether differences in virulence or variation in environmental conditions, or both cause variation of field population densities of *M. chitwoodi*. This is still under investigation.

Acknowledgements

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Chapter 3

Tuber yield, quality and infestation of potato genotypes resistant to *Meloidogyne chitwoodi*

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To be submitted

As part of developing a resistance test for potatoes to *Meloidogyne chitwoodi*, also growth, yield, quality loss and tuber infestation levels of 8 genotypes, 7 with a single resistance gene to *M. chitwoodi* and one, MDG2, with resistance to *Globodera pallida*, were studied in four greenhouse experiments using cv. Desiree as susceptible control. Plants were inoculated with ranges of densities from 0.0625 to 256 J2(g dry soil)⁻¹ in log series of 2^x. Haulm height, fresh tuber weight, tuber quality and tuber infestation were recorded. Harvested tubers of experiment 2 were stored for 240 - 300 days to estimate actual tuber infestation at planting.

Plants showed a normal logistic growth curve in all experiments. Plant height was positively affected with increasing population densities (P_i) and negatively with decreasing pot size. In general, growth was delayed at higher $P_i \geq 32$ J2(g dry soil)⁻¹. Tuber yield was not affected in four out of seven genotypes with resistance to *M. chitwoodi*; they can be considered as tolerant. Three genotypes and cv. Desiree showed minimum yields (m) < 0.8, the latter varying between 0.67 and 0.80 over experiments and pot size. Quality, expressed as tuber knot-index, used for ware potatoes, was well below 10 for all genotypes but not for cv. Desiree and MDG2. The fraction of clean tubers was significantly increased compared to cv. Desiree. The nematodes from tubers of cv. Desiree ($P_{f_{\text{tubers}}}$) in Experiment 2 after 240-300 days of storage showed no regression with the P_i and averaged 0.35 J2(g dry soil)⁻¹, while all tested genotypes provided ca. 0.002 J2(g dry soil)⁻¹.

Keywords: Clean tubers, growth, root-knot nematodes, tolerance.

Introduction

Meloidogyne chitwoodi and *Meloidogyne fallax* (Karssen, 1995, 1996) are quarantine pests in the EPPO region and pose a serious threat to seed potato growers in The Netherlands (Been *et al.*, 2007). Aiming at the same successful management approach used to control the potato cyst nematodes (PCN) in The Netherlands, the use of resistant cultivars (Been & Schomaker, 1998) is envisaged, and Dutch breeders are engaged in the development of potato genotypes resistant to *M. chitwoodi*. These genotypes, with a single resistant gene, have been reported to possess high level of resistance (Norshie *et al.*, 2011; Teklu *et al.*, 2016).

In addition to resistance, also yield, quality loss and tuber infestation are of major interest when these resistant potatoes are grown. The quantitative information available concerning the effect of *M. chitwoodi* on yield of potato cultivars is often contradicting. Both Griffin (1985) and Ingham *et al.* (2007), reported that *M. chitwoodi* did not affect potato yield significantly. By contrary, Pinkerton & Santo (1986), reported a yield reduction of up to 10-tonne ha⁻¹, when compared to nematicide treated plots, while Viglierchio (1987) reported tuber yield reduction by *M. chitwoodi* only at very high, but not quantified, P_i 's. In general, no yield losses have been reported in The Netherlands caused by *M. chitwoodi* under field conditions while Norshie *et al.* (2011) reported a 12% fresh tuber weight loss of cv. Desiree in pot experiment.

However, the economically most significant damage of *M. chitwoodi* is the quality loss inflicted to tuber forming crops *e.g.* potatoes, carrots and black salsify (Wesemael & Moens, 2008; Norshie *et al.*, 2011; Heve *et al.*, 2015). Generally, zero tolerance applies when *M. chitwoodi* is detected in seed potato lots (Ingham *et al.*, 2007; EPPO/OEPP, 2013; King & Taberna, 2013). Australia allows 2% damage in seed potatoes by root-knot nematodes in general as the country is free of *M. chitwoodi* infestations (Anonymous, 2007).

However, there are acceptable limits for industrial processing. In the US, a potato lot will be rejected when 5-15% of the tubers have to be discarded because of *M. chitwoodi* deformations (Ingham *et al.*, 2000, 2007; King & Taberna, 2013). In The Netherlands, infested tubers with a tuber-knot index (TKI) not exceeding 10 are accepted for ware potatoes (Visser & Korthals, 2004). This index is relaxed to 20 at times of shortages.

Yield and quality reduction associated with nematodes is mainly dependent on the host sensitivity of the crop and the initial nematode population density (P_i) at planting (Van Riel, 1993; Wesemael & Moens, 2008; Norshie *et al.*, 2011). The relation has to be investigated using a range of population densities (Schomaker & Been, 2013). This paper combines the data on fresh tuber weight, quality and tuber infestation of Norshie *et al.* (2011) and 3 subsequent experiments carried out to develop a resistance test for *M. chitwoodi* and potatoes (Teklu *et al.*, 2016). The experiments include two starch and six ware potato genotypes with various sources of resistance against *M. chitwoodi*. The findings should provide some basic insights regarding yield loss, quality loss and tuber infestation levels of the newly developed resistant genotypes. This research is part of a larger research project (MeloResist), a cooperation between Wageningen University & Research and Dutch potato

breeders, to develop a standardized test for the estimation of resistance of potato genotypes against *M. chitwoodi*.

Materials and Methods

Four glasshouse experiments were carried out between 2010 and 2014 (Table 3.1). Eight potato genotypes, resistant to *M. chitwoodi*, and one genotype resistant to *G. pallida* only were compared once or more to cv. Desiree at ranges of 11-13 initial population densities. The “Smakt”, Mc31 population, which is the population used in the European DREAM project (EU QLRT-1999-1462) and chosen as the Dutch standard test population so far, was used in this research. Each P_i had 4-5 replications per experiment and different pot sizes were used (Teklu *et al.*, 2016). A mixture of silver sand (60%), hydro-grains (30%) and clay powder (10%), to which 1 g NPK (12:10:18) fertilizer was added per kg was used. The pots were inoculated by injecting juvenile suspensions randomly distributed into 4 to 20 narrow channels, depending on pot size, from top to bottom of the pots. A cylindrical piece of tuber with a single sprout was planted at 6 cm depth. The experiments lasted 13-16 weeks. Pots were watered and rotated every week to diminish positional effect in the glasshouse. Moisture content of the soil was maintained at 12-15%. For a full description of the setup of these experiments consult Teklu *et al.* (2016).

Table 3.1. Overview of experimental set up used, potato genotypes tested, source of resistance, parent material and the breeding company. Experiment 1 by Norshie *et al.* (2011). Experiment 2 to 4 by Teklu *et al.* (2016). The cultivar Desiree was used as a control in all experiments.

Year	Exp.	Location	Pot size (kg)	Rep	Densities	Genotypes	Source of resistance	Resistant parent	Breeding company
2010	1	Wageningen	5	5	11	ARo4-4096	<i>S. bulbocastanum</i>	BLBC 398.89	Agrico
						ARo4-4098	<i>S. bulbocastanum</i>	BLBC 398.89	Agrico
						0.5-256 ARo4-4107	<i>S. hougasii</i>	HOU M 94-110-2	Agrico
2011	2	Wageningen	10	4	12	ARo4-4096			
						ARo5-4044	<i>S. hougasii</i>	HOU M 94-110-2	Agrico
						Ka-2006/2217	<i>S. hougasii</i>	M 94-110-2	Averis
						0.125-128 Ka-2007/1312	<i>S. fendleri</i>	M 94-125-1	Averis
2012	3	Wageningen	10, 5, 2	4, 4, 5	12	2011M1	<i>S. fendleri</i>	M 94-144-1	HZPC
						0.125-128 MDG2	<i>S. vernei</i>	AM 78-3778	KWS
2014	4	Wageningen	3	5	13	ARo4-4096			
						0.0625-128 2011M1			

Agrico, PB 70, 8300 AB Emmeloord, The Netherlands; Averis Seeds B.V., Kweekinstituut Karna, Valtherblokken Zuid 40, 7876TC, Valthermond, The Netherlands; HZPC Holland B.V., Research & Development, PB 2, 9123 ZR Metslawier, The Netherlands; KWS POTATO B.V., Johannes Postweg 8, 8308 PB Nagele, The Netherlands.

Measurements and observations

Height as growth indicator

Height of plants was measured every week until growth stopped and at least three consecutive measurements produced the same height. Measurements were taken from a fixed point of an inserted plastic peg in the pot to the tip of the potato stem.

Fresh tuber weight (FTW)

Harvested tubers were placed in meshed bags, rinsed with a thin film of water to remove any adhering soil and were allowed to dry overnight in the glasshouse at 20°C, after which the FTW per pot was measured.

Tuber knot-index and proportions of clean tubers

Tuber knot-index and proportions of clean tubers were assessed by visual inspection and scoring of the potato skin after peeling the first 5 mm of the skin (Viaene *et al.*, 2007). Tubers were scored and classified according to Visser & Korthals (2004) to calculate the tuber-knot index and the proportion of clean tubers; the former is used by the Dutch potato processing industry to decide suitability for industrial processing. For the details of the methodology refer to Teklu *et al.* (2017). As zero tolerance applies in seed potatoes, the proportion of clean tubers (class zero: no symptom on the outer skin and no egg masses detected inside the tuber) was estimated separately to assess their quality as seed tubers. Out of the total tubers harvested per pot only those > 25 mm (except Experiment 4 > 15 mm) diameter were scored to avoid bias due to tuber size.

Tuber infestation levels ($P_{t_{\text{tubers}}}$)

Harvested tubers of experiment 2 were stored for 240-300 days to estimate actual tuber infestation at planting. Throughout the storage period, a temperature of 7°C and humidity of 99% were maintained. Due to limited capacity of the mist chamber, replications 1 and 2 were processed after 240 days and replications 3 and 4 after 300 days. Tubers were exposed to 20°C for 2 weeks to harden their skin. Potato tubers were peeled 5 mm deep (Viaene *et al.*, 2007). The peel was then cut into 1 cm² pieces and placed on 20 cm diameter, 425 µm extraction sieves. The sieves were put on 25 cm diameter extraction dishes and kept in the spray-mist chamber for 7 weeks, while hatched J2 were collected and counted every 7 days. The logistic hatching curve of the susceptible cv. Desiree was used as a reference for the genotypes to monitor the hatching process and to determine the time to stop the hatching process.

Data analysis and Modelling

Scripts for data analysis and modelling were written using the Tinn-R editor version 4.0.2.1 and run using the statistical environment R-3.2.2. Regression analysis (Ordinary Least Squares) was carried out to describe the growth of the potato plants and the effect of P_i on fresh tuber weight (FTW). Starting parameters were estimated directly from the data and, in nonlinear regression analysis, the standard errors were estimated using the inverse of the Hessian matrix. If relevant, estimated parameters of genotypes were compared and tested at a 5% level of uncertainty using Least Significant Difference (LSD).

Tuber quality for seed (Fo and Fo1) and tuber infestation ($P_{f_{tuber}}$) pot^{-1} expressed as $\text{J2(g dry soil)}^{-1}$ using the total weight of the pot were also estimated. To estimate $P_{f_{tuber}}$ per genotype/cultivar, replicates were first log transformed and the averages back transformed. As the number of zero nematode counts depends on pot and sample size, they were considered to be small numbers and handled as described in Teklu *et al.* (2017). Per (genotype/cultivar $\times P_i$), replicates were averaged and plotted against ranges of P_i to study the pattern and choose the best model.

3

Plant height

Effect of P_i on plant height in time was derived from the logistic growth model Equation 1:

$$F_h(t) = \frac{\lambda}{1 + \exp(-\beta(t - \alpha))} \quad (1)$$

- λ the maximum plant height in cm
- β the relative maximum growth rate
- α time to reach $0.5 \times \lambda$
- t Growth period (days)

Effect of P_i on fresh tuber weight

Seinhorst's (1965, 1998) yield loss model was used to quantify the effect of P_i on fresh tuber weights (FTW). Parameters: the tolerance limit (T) in $\text{J2(g dry soil)}^{-1}$ and the relative minimum FTW, (m), were estimated.

$$\text{FTW} = Y_{\max} \times m + (1 - m)0.95^{P_i/T-1} \quad (2)$$

Tuber quality

To quantify the tolerance, T_{qual} , of the genotypes for quality damage, the maximum P_i was estimated where Fo, the fraction of tubers in class o, was larger than 0.9. Class o comprises the tubers without external and internal symptoms.

$$T_{qual} = \max(P_i [F0 > 0.9]) \quad (3)$$

Tuber infestation levels $P_{f_{tubers}}$

Equation 4, was fitted to the number of hatched J2 originating from the peel of the tubers. As $P_{f_{tubers}}$ was not affected by the P_i , averages of log transformed numbers were calculated and back transformed.

$$F_j(t) = \frac{C}{1 + \exp(-B(t - A))} \quad (4)$$

The interpretation of the parameter estimates was now:

- C maximum cumulative hatch J2(g dry soil)⁻¹
 B the relative maximum hatching rate
 A time to reach $0.5 \times C$

Results**Growth**

The growing period of the potato plants varied from 13-16 weeks between experiments. The logistic model (Equation 1) fitted well to the data of the four experiments at all nematode densities with $0.911 < R^2 < 0.999$. As an example, results of experiment 2 are summarized in Table 3.2. In 68% of the fitted lines, the maximum plant height λ at $P_i = 32\text{-}128 \text{ J2(g dry soil)}^{-1}$ was increased compared to that at $P_i = 0$ (Figure 3.1). Similarly, 60% of the plants at $P_i = 32\text{-}128 \text{ J2(g dry soil)}^{-1}$ needed extra time D , in total $2 \times \alpha + D$, to reach their maximum height compared to plants at $P_i = 0$. Over all experiments, D varied between 10 and 50 days. In experiment 2, D was the largest and varied between 40 and 50 days.

For all genotypes and cv. Desiree, decrease of pot size affected plant height (λ) and (α), time to reach $0.5 \times \lambda$, negatively (Table 3.3). In the 10 kg pots, the relative maximum growth rate (β) is smaller than that in the 2 kg pots. Consequently, plants grown in these pots took more time to reach their maximum height (λ) and senescence occurred later, compared to plants in smaller pots.

Fresh tuber weight

In 10 out of 21 analyses, no regression was found between P_i and fresh tuber weight (FTW) (Table 3.4). Over all pot sizes and experiments an average minimum fresh tuber yield of 0.78 and 0.84 was obtained for cv. Desiree and genotype MDG2, respectively. Genotype AR04-4096 and 2011M1 tested 4 and 3 times, respectively, and in different pot sizes, showed no regression ($m = 1$). For genotypes AR05-4044, Ka-2006/2217, Ka-2007/1312 grown in 10 kg pots (Experiment 2), m was decreased (Figure 3.2). All remaining genotypes showed no decreased FTW. Absolute FTW harvested was directly proportional to pot size and a linear correlation was established (Figure 3.3): $Y_{\max} = 15.23 + 28.3 \times \text{pot size}$ ($R^2 = 0.99$). No correlation was established between pot size and T or m .

Table 3.2: Parameter estimates of the logistic function; Equation 1. $F_h(t) = \lambda / (1 + \exp(-\beta(t - \alpha)))$ used to fit the data of plant height of Experiment 2, displayed in Figure 3.1. Potato growth at $P_i = 0$ was compared to that of plants at $P_i = 32, 64$, and $128 \text{ J2(g dry soil)}^{-1}$ of *Meloidogyne chitwoodi*. Where: λ = maximum height in cm; β = relative maximum growth rate; α = time for the plants to reach $0.5 \times \lambda$ in days; SE = standard error of the parameters; R^2 = coefficient of determination and df = degrees of freedom.

Genotype/CV	Exp	Pot size (kg)	P_i	λ	β	α	SE_λ	SE_β	SE_α	R^2	df
AR04-4096	2	10	0	82	0.14	24.6	0.58	0.01	0.60	0.997	9
AR04-4096	2	10	32	122*	0.11	44.7	0.91	0.004	0.34	0.999	9
AR04-4096	2	10	64	122*	0.12	45.7	0.98	0.005	0.35	0.999	9
AR04-4096	2	10	128	124*	0.12	44.5	0.92	0.004	0.33	0.999	9
AR05-4044	2	10	0	113	0.12	28.9	1.05	0.01	0.59	0.997	9
AR05-4044	2	10	32	149*	0.08	55	1.94	0.003	0.55	0.999	9
AR05-4044	2	10	64	138*	0.08	48.3	2.16	0.005	0.71	0.998	9
AR05-4044	2	10	128	132*	0.08	54.7	2.74	0.004	0.90	0.998	9
Ka-2006/2217	2	10	0	134	0.08	35.8	2.17	0.01	0.86	0.995	9
Ka-2006/2217	2	10	32	157*	0.07	55.5	6.62	0.01	1.96	0.994	9
Ka-2006/2217	2	10	64	161*	0.08	49	1.91	0.003	0.54	0.999	9
Ka-2006/2217	2	10	128	136	0.07	64.7	2.67	0.002	0.82	0.999	9
Ka-2007/1312	2	10	0	106	0.11	28.6	2.33	0.02	1.42	0.983	9
Ka-2007/1312	2	10	32	153*	0.08	53.3	2.06	0.003	0.59	0.999	9
Ka-2007/1312	2	10	64	151*	0.10	44	1.08	0.003	0.33	0.999	9
Ka-2007/1312	2	10	128	149*	0.09	49.6	1.11	0.003	0.33	0.999	9
Desiree	2	10	0	114	0.10	32.6	1.46	0.01	0.74	0.995	9
Desiree	2	10	32	160*	0.07	67.2	6.33	0.005	1.55	0.997	9
Desiree	2	10	64	145*	0.07	52.8	3.71	0.005	1.17	0.997	9
Desiree	2	10	128	145*	0.08	52.1	2.93	0.005	0.89	0.997	9

* Significantly different parameter values at $P = 0.05$ when compared to $P_i = 0 \text{ J2(g dry soil)}^{-1}$.

Table 3.3. Parameter estimates of the logistic function; Equation 1. $F_h(t) = \frac{\lambda}{1 + \exp(-\beta(t - \alpha))}$ used to fit the data of plant height of Experiment 3, with three different pot sizes (2, 5 and 10 kg) at $P_i = 0 \text{ J2(g dry soil)}^{-1}$ of *Meloidogyne chitwoodi*.

Genotype/cv	Exp	Pot size (kg)	P_i	λ	β	α	se_λ	se_β	se_α	R^2	df
2011M1	3	2	0	83.4	0.173	23.04	0.56	0.0095	0.3096	0.999	7
2011M1	3	5	0	104.8*	0.125*	30.99*	4.32	0.0369	1.9123	0.979	7
2011M1	3	10	0	122.7*	0.088	34.56*	5.17	0.0146	2.0988	0.986	7
Desiree	3	2	0	84.3	0.097	28.64	2.82	0.0171	1.6902	0.984	7
Desiree	3	5	0	109.8*	0.086	36.45*	7.12	0.0195	3.2170	0.973	7
Desiree	3	10	0	147.4*	0.067	42.18*	13.66	0.0140	4.5424	0.973	7
MDG2	3	2	0	83.5	0.161	26.30	0.99	0.0151	0.5148	0.996	7
MDG2	3	5	0	95.5*	0.141*	29.28*	1.69	0.0184	0.7860	0.994	7
MDG2	3	10	0	129.0*	0.092*	35.94*	4.97	0.0136	1.8987	0.988	7

* Significant difference with previous data point

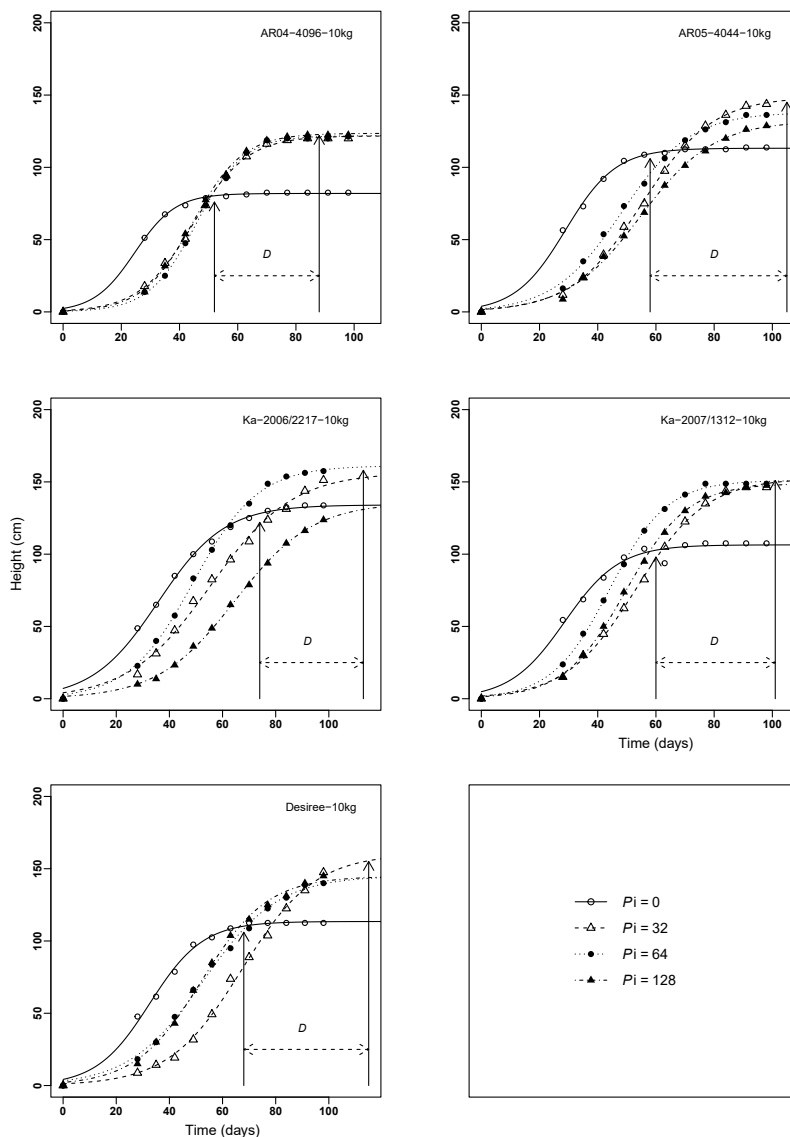


Figure 3.1. The logistic growth model: Equation 1. $E_i(t) = \frac{\lambda}{1 + \exp(-\beta(t - \alpha))}$ fitted to the plant height of potato genotypes and cv. Desiree from Experiment 2. Plant height at $P_i = 0$ is compared to that of $P_i = 32, 64$ and 128 $J_2(g \text{ dry soil})^{-1}$ of *Meloidogyne chitwoodi* in time. D = extra time needed to reach λ when plants at $P_i = 0$ are compared to those at $P_i = 128$.

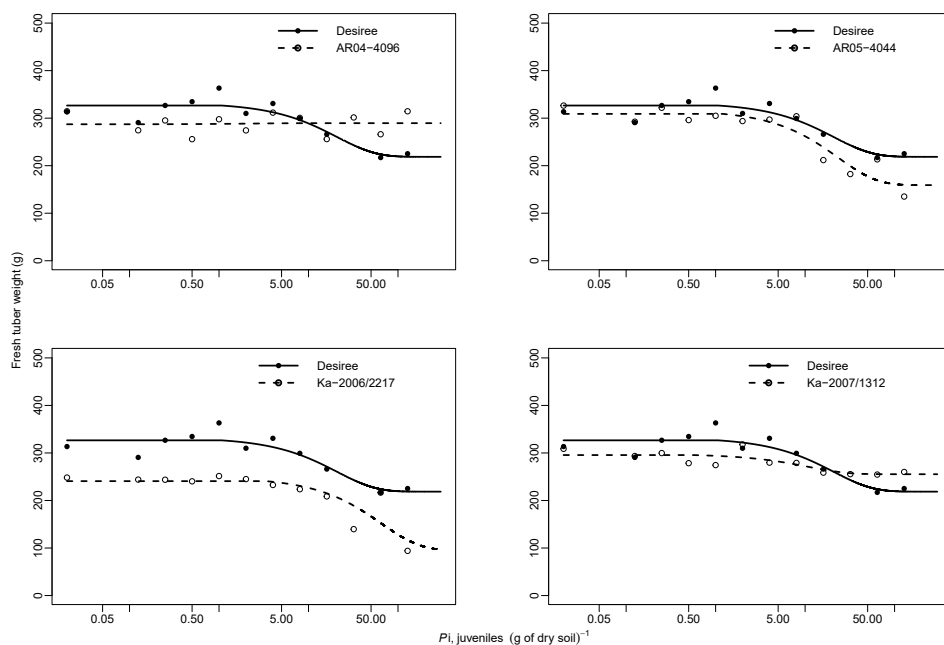


Figure 3.2. Seinhorst model Equation 2: $FTW = Y_{max} \times m + (1-m)0.95^{P_i/T-1}$ for yield loss fitted to describe the relation between the initial population density (P_i) of *Meloidogyne chitwoodi* and the fresh tuber weight (FTW) for four genotypes and cv. Desiree of Experiment 2.

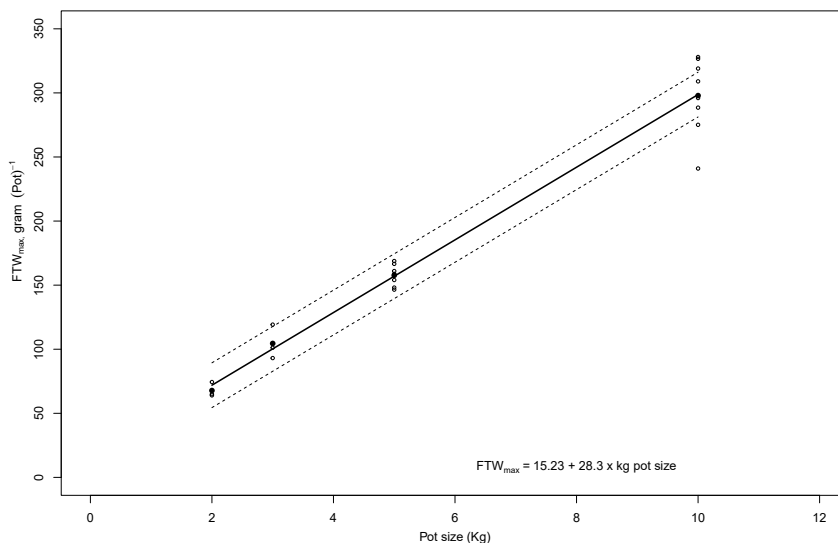


Figure 3.3. Linear relation between pot size and Y_{max} , maximum fresh tuber weight, (FTW_{max}), the yield at $P_i = 0.12$ (g dry soil) $^{-1}$ of *Meloidogyne chitwoodi* irrespective of genotype or cv. Desiree. $R^2 = 0.99$. Open dots (○) data points, closed dots (●) mean Y_{max} . Broken lines are the 2.5 and 97.5 quantile of the regression line.

Table 3.4. Parameter estimates for the relation between P_i of *Meloidogyne chitwoodi* and FTW according to Equation 2: $FTW = Y_{max} \times m + (1-m)0.95^{P_i/T-1}$ and the relation between P_i and Fo. T = tolerance limit, J_2 (g dry soil) $^{-1}$, m = minimum yield, Y_{max} = g maximum fresh tuber weight (FTW), SE = standard error, R^2 = coefficient of determination. Regr. no regression found '-'; regression found '+' Equation 3; $T_{qual} = \max(P_i [Fo > 0.90])$; Fo = the fraction of tubers in class zero (no external or internal symptoms);

Genotype/cv	Exp	Pot size (kg)	T	m	Y_{max}	SE_T	SE_m	$SE_{Y_{max}}$	R^2	df	Regr	T_{qual}
ARo4-4107	1	5	NA	1.00	158.8	NA	0.028	4.42	NA	9	-	32
ARo4-4098	1	5	NA	1.00	168.8	NA	0.029	4.97	NA	9	-	32
ARo4-4096	1	5	NA	1.00	166.6	NA	0.031	5.15	NA	9	-	16
Desiree	1	5	3.98	0.72	161.0	7.30	0.192	10.13	0.35	8	+	≤ 0.5
ARo4-4096	2	10	NA	1.00	288.5	NA	0.024	6.97	NA	10	-	128
ARo5-4044	2	10	1.20	0.51	309.0	0.69	0.091	12.70	0.84	9	+	0.25
Ka-2006/2217	2	10	3.35	0.40	241.0	NA	0.400	13.91	0.66	9	+	>128
Ka-2007/1312	2	10	0.56	0.86	296.0	NA	0.040	7.23	0.58	9	+	2
Desiree	2	10	1.07	0.67	326.6	0.59	0.064	10.32	0.79	9	+	≤ 0.125
2011M1	3	10	NA	1.00	275.1	NA	0.026	7.12	NA	10	-	0.25
MDG2	3	10	0.027	0.74	328.0	0.017	0.044	18.01	0.77	9	+	≤ 0.125
Desiree	3	10	0.015	0.70	319.0	0.010	0.062	25.73	0.66	9	+	≤ 0.125
2011M1	3	5	NA	1.00	148.1	NA	0.026	3.87	NA	10	-	0.125
MDG2	3	5	NA	1.00	146.5	0.107	0.043	6.30	0.23	10	-	≤ 0.125
Desiree	3	5	0.003	0.80	154	0.006	0.098	17.45	0.16	9	+	≤ 0.125
2011M1	3	2	NA	1.00	64.9	NA	0.026	1.71	NA	10	-	0.5
MDG2	3	2	0.112	0.78	74.4	0.115	0.051	4.01	0.65	9	+	≤ 0.125
Desiree	3	2	0.195	0.79	64	0.150	0.051	2.77	0.64	9	+	≤ 0.125
ARo4-4096	4	3	NA	1.00	93.1	2.660	0.027	2.55	NA	11	-	NA
2011M1	4	3	NA	1.00	119.2	NA	0.015	1.84	NA	11	-	NA
Desiree	4	3	0.013	0.78	101.2	0.010	0.048	5.70	0.71	10	+	NA

Tuber quality

Clean tubers (F0: No external or internal symptoms)

The estimates of Fo for Equation (3) concerning the proportion of clean tubers are listed in Table 3.5. Quality was affected at the lowest P_i used in each experiment for cv. Desiree and MDG2, the latter having resistance against *G. pallida*. All resistant genotypes performed better, but eventual presence of *M. chitwoodi* was demonstrated. The proportion of clean tubers was 91% for all genotypes, except for 2011M1 with 53%. Desiree and MDG2 yielded no clean tubers, except some rare exceptions were potatoes surfaced the soil and were not infected.

Tuber-knot index (TKI)

In general, external symptoms were drastically reduced as is shown in Figure 3.4 where potatoes from genotypes ARo4-4096, ARo5-4044 & Ka-2007/1312 at the highest density of 128 J_2 (g dry soil) $^{-1}$ are compared to tubers from cv. Desiree at 0, 0.25 & 0.5 J_2 (g dry soil) $^{-1}$. All resistant genotypes, except 2011M1, had TKI scores < 10 at all initial population densities. Both cv. Desiree and genotype MDG2 produced TKI values exceeding 20 at all P_i values.

Only genotype 2011M1 showed increasing TKI value at increasing P_i in Experiment 3 with a highest TKI value a score of 47 at $P_i = 8 \text{ J}_2(\text{g dry soil})^{-1}$ at 5 kg pot. By contrast, in Experiment 4 2011M1 produced a TKI values below 10 at all P_i values (Table 3.5).

Table 3.5. The tuber knot-index of *Meloidogyne chitwoodi* according to Visser & Korthals (2004) and the proportion of Fo (no external or internal symptoms), F1 (no external symptoms) and Fo+1, per genotype averaged over all P_i 's.

Genotype/cv	Exp	Pot Size(Kg)	TKI	Proportion of		
				Fo	F1	Fo+1
AR04-4107	1	5	1.33	0.88	0.08	0.92
AR04-4098	1	5	0.27	0.96	0.02	0.97
AR04-4096	1	5	1.57	0.87	0.07	0.95
Desiree	1	5	70.21	0.09	0.00	0.18
AR04-4096	2	10	0.38	0.99	0.00	0.96
AR05-4044	2	10	0.71	0.96	0.00	0.93
Ka-2006/2217	2	10	0.00	1.00	0.00	1.00
Ka-2007/1312	2	10	1.42	0.94	0.00	0.91
Desiree	2	10	57.18	0.11	0.00	0.16
2011M1	3	10	9.16	0.48	0.02	0.51
MDG2	3	10	67.30	0.11	0.00	0.12
Desiree	3	10	63.62	0.14	0.02	0.18
2011M1	3	5	16.62	0.46	0.00	0.49
MDG2	3	5	61.38	0.09	0.00	0.14
Desiree	3	5	66.83	0.11	0.00	0.14
2011M1	3	2	12.19	0.50	0.00	0.52
MDG2	3	2	69.51	0.08	0.00	0.08
Desiree	3	2	76.61	0.08	0.00	0.09
AR04-4096	4	3	0.00	1.00	0.00	1.00
2011M1	4	3	0.66	0.98	0.00	0.95
Desiree	4	3	28.52	0.19	0.00	0.28

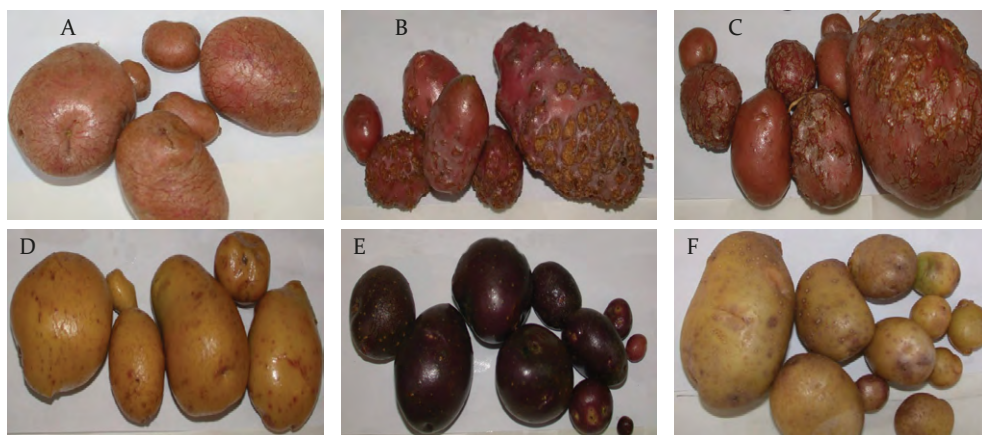


Figure 3.4 Quality damage caused by of *Meloidogyne chitwoodi* on the susceptible cv. Desiree (top row) and resistant genotypes of Experiment 2 (bottom row). A, B, C: cv. Desiree at $P_i = 0, 0.25$ and $0.5 \text{ J}_2(\text{g dry soil})^{-1}$, respectively; D, E, F: genotypes AR04-4096, AR05-4044 and Ka-2007/1312 at $P_i = 128 \text{ J}_2(\text{g dry soil})^{-1}$.

Final population from tubers $P_{f_{\text{tubers}}}$ (Experiment 2)

The logistic equation (Equation 4) fitted well ($R^2 > 0.98$ for all P_i 's) to the number of hatched J2 of *M. chitwoodi* from tubers of cv. Desiree, but not to those of the four tested resistant genotypes, due to the small numbers present in the tubers which made curve fitting impossible.

The output of the logistic regression for cv. Desiree and all resistant genotypes of Experiment 2 for parameter C are presented in Table 3.6. The estimates of C are considered the $P_{f_{\text{tuber}}}$ of cv. Desiree. As no regression was found between P_i and $P_{f_{\text{tuber}}}$ for cultivar Desiree (Figure 3.5), all data were pooled and the average $P_{f_{\text{tuber}}}$ J2(g dry soil) $^{-1}$ on cv. Desiree was estimated to be 0.35. This value equates to 1.9% of the total P_f from the roots, soil and tubers combined of cv. Desiree. Compared to cv. Desiree, the tested genotypes were only marginally infested; $P_{f_{\text{tuber}}}$ was 0.002 J2(g dry soil) $^{-1}$, 2.1% of the total P_p the same percentage as on the tubers cv. Desiree (Table 3.6). Compared to Desiree, tuber infection level of the resistant genotypes was less than 1%, with the exception of Ka-2007/1312 with 7.6%.

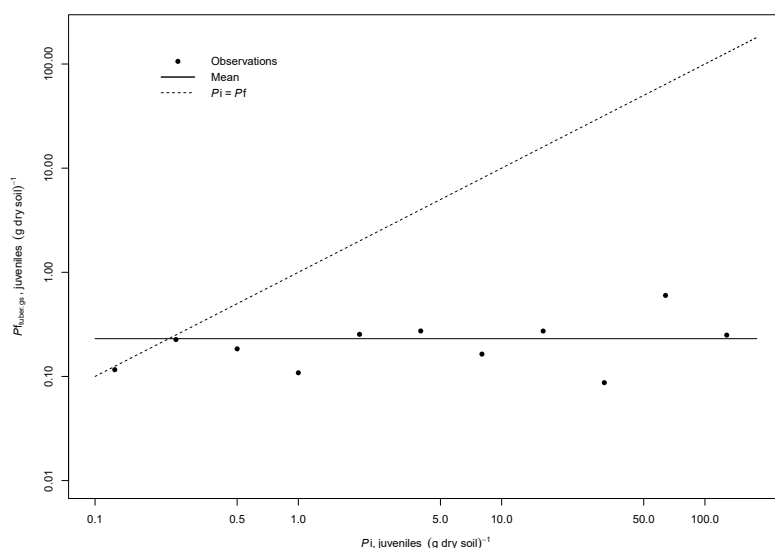


Figure 3.5. The relation between P_i of *Meloidogyne chitwoodi* and parameter C , the maximum cumulative hatch, on cv. Desiree after storage. The diagonal straight fine broken line is the equilibrium line ($P_i = P_{f_{\text{tuber}}}$), the solid fitted line is the mean of parameter C , over the different P_i 's.

Table. 3.6: Cumulative hatch after 7 weeks in the mist-chamber of of *Meloidogyne chitwoodi* J2(g dry soil)⁻¹, indicating tuber infestation levels from the potato tubers of cv. Desiree compared to the resistant potato genotypes. P_i = initial population density J2(g dry soil)⁻¹.

P_i	Cumulative hatch C, J2(g dry soil) ⁻¹				
	Desiree	AR04-4096	AR05-4044	Ka-2006/2217	Ka-2007/1312
0.125	0.116	0.0050	0.0000	0.0000	0.0000
0.25	0.226	0.0043	0.0000	0.0005	0.0017
0.5	0.184	0.0000	0.0000	0.0010	0.0008
1	0.108	0.0000	0.0000	0.0021	0.0026
2	0.254	0.0000	0.0000	0.0000	0.0023
4	0.273	0.0018	0.0000	0.0000	0.0026
8	0.164	0.0000	0.0011	0.0032	0.0007
16	0.273	0.0000	0.0018	0.0000	0.0030
32	0.087	0.0033	0.0000	0.0019	0.1690
64	0.600	0.0007	0.0008	0.0035	0.0034
128	0.249	0.0011	0.0000	0.0000	0.0055

3

Discussion

Growth

Growth of the haulm height was logistic regardless of genotype, but differed in the maximum reached per genotype and pot size. No specific above ground symptoms were observed in any genotype including cv. Desiree, in contrast to other crops (Wesemael & Moens, 2008; EPPO/OEPP, 2013). Thinner and longer plants noticed in this experiment at higher $P_i \geq 32$ J2(g dry soil)⁻¹, were also reported by Norshie *et al.* (2011) and Heve *et al.* (2015) in potato and carrots and *M. chitwoodi*, respectively. Seinhorst & Den Ouden (1971) also reported this for potato in combination with *Globodera* spp. Increasing crop height with increasing pot size can be attributed to the increasing volume of soil accommodating larger plants, resulting also in higher fresh tuber yields.

Fresh tuber weight

The fresh tuber weights of cv. Desiree and genotype MDG2 (without resistance to *M. chitwoodi*), showed a reduction at the highest nematode densities in all experiments in which they were tested. In addition, AR05-4044, Ka-2006/2217 and Ka-2007/1312 suffered yield losses. This proves that *M. chitwoodi* can also reduce the yield of potatoes and partly supports the findings of Pinkerton & Santo (1986), who reported a yield reduction of up to 10 tonne ha⁻¹, when control plots were compared to nematicide treated plots. These plots would also have benefited from ca. 100 kg extra N due to the soil fumigation and only part of the yield difference might be attributed to *M. chitwoodi*. Viglierchio (1987) reported tuber yield reduction by *M. chitwoodi* only at very high, but no specific initial population densities were reported. In the experiments carried out yield reduction becomes visible at the highest densities. Generally, these high densities of *M. chitwoodi*, as used in

the reported experiments are not available under field conditions in spring. The highest infestations so far recorded were about 3-5 J2(g dry soil)⁻¹ in The Netherlands (Molendijk, pers.comm.). This might explain that under field conditions, including all the variations caused by differences in growing conditions, the probability that yield reduction will be encountered or noticed is minimal.

Genotypes AR04-4096 and 2011M1, which were tested several times, and AR04-4098, and AR04-4107, tested only once, had $m = 1$ and can be considered tolerant to *M. chitwoodi* at all population densities tested. Tolerance in these genotypes also indicates that root growth was not reduced and that these resistant potato genotypes, when used in the field, are likely to provide maximum effect on the population densities present.

Quality deterioration

Seed potatoes

Tuber infestation was observed even at the lowest P_i used 0.0625 J2(g dry soil)⁻¹ in the four experiments on both cv. Desiree and MDG2 with > 92% of the tubers infected over all P_i values. It indicates that a tolerance limit for clean potatoes, if it exists, has to be found at an even lower population density. A tolerance limit ≤ 0.004 J2(g soil)⁻¹ for quality damage of potato was reported (Ingham *et al.*, 2000; Pinkerton & Santo, 1986). This was mainly for cultivars, which lack resistance for *M. chitwoodi*, equivalent to cv. Desiree and MDG2 in this research. However, this was not the case with the resistant genotypes, with 91% of the tubers free from infestation over all P_i levels. As zero tolerance applies for *M. chitwoodi* in seed potatoes, the use of these resistant potatoes for seed when grown on infected soil still needs further investigation. As in the controlled environment of a glasshouse, the resistant genotype showed a remarkable improvement in quality and at lower densities quality loss was not always demonstrable, application in the field below a certain population threshold might be feasible. In a first pilot experiment with a selection of these genotypes on a farmers field no quality loss was observed at all, while on a field where populations during winter were kept comparably high with a susceptible green manure crop, some quality damage was registered (Teklu *et al.*, unpubl.).

Ware potatoes for industrial processing

By contrast, the tuber-knot index for all genotypes except 2011M1 was below the minimum threshold level (< 10) at all population densities tested and therefore acceptable for industrial processing. For genotype 2011M1 the TKI value is (< 10) at P_i value of ≤ 2 J2(g dry soil)⁻¹ and are only acceptable for processing during time of shortages at P_i values ≥ 2 J2(g dry soil)⁻¹. The noticeably higher TKI of 2011M1 as compared to the rest would most likely be due to a slightly lower resistance (Teklu *et al.*, 2016) and the effect of higher population densities at tuber formation (Teklu *et al.*, 2017). The susceptible cv. Desiree and genotype MDG2, which does not contain *M. chitwoodi* resistance, had TKI scores > 20 at almost all densities and would not be accepted for industrial processing.

Tuber infestation levels ($P_{f_{\text{tubers}}}$)

Obviously, the J2 obtained from the tubers are part of the P_f . However, they do not remain in the soil, but are transported off field with the harvested tubers. While tuber infestation of starch potatoes is ignored, and a certain level is tolerated in ware potatoes (TKI either equal or smaller than 10 or 20, depending on demand and supply), seed will be rejected when detection occurs. In Experiment 2, J2 of *M. chitwoodi* were found in peel of potato tubers of both cv. Desiree and the resistant genotypes after 240-300 days of storage, although in lower levels, on average 2%, compared to the numbers in the soil and roots after harvest of the genotype or cultivar. Compared to tubers of cv. Desiree, the densities in the resistant potatoes were reduced by more than 90%, and extremely low. It is not known whether this low $P_{f_{\text{tubers}}}$ is the result of low densities at tuber formation or is related with a certain maximum carrying capacity of the tubers.

As only densities after storage were estimated, due the limited numbers of tubers available, we do not know whether *M. chitwoodi* numbers increased, declined or were stable during storage. According to Teklu *et al.* (2018) a storage temperature of 4°C reduces $P_{f_{\text{tubers}}}$, at 8°C population densities remain the same for 120 days and then start to decline. At 12°C, during the first 60 days, densities even increase, probably by further egg laying of the matured females, after which numbers remain unchanged until 240 days, the maximum storage time used. The surviving J2 on cv. Desiree, independent on storage temperature, were as viable as freshly hatched ones (Teklu *et al.*, 2018). The 7°C in this experiment suggest that there might be a slight reduction in numbers compare to harvest and it is likely that they will multiply under the next potato crop.

The method of visual inspection of 200 tubers per 10 tonnes of potato (EPPO, 2006) seems not very effective to trace the infection levels obtained in the resistant genotypes. The recently introduced molecular techniques with sensitivity of 1 matured female might help (De Haan *et al.*, 2014) but detection currently in the susceptible cultivars is mostly based on one potato with one female detected in the whole lot (Jan Luimes, pers.comm.). So infected lots will slip through inspection and reach new fields.

When we try to estimate the risk of using infected susceptible tubers after storage as seed in the field situation we can make the following assumptions:

- i) Seed rate is 4 tubers in m^2 (Van der Zaag, 1992); seed tuber size of 35- 45 mm diameter, at the mid of the oval shape;
- ii) Depth of tilth is 25 cm, consequently a volume of $100 \times 100 \times 25 \text{ cm}^3$ per m^2 is available;
- iii) One (1 cm^3) soil is equivalent to 1.3 g soil;
- iv) Based on four tubers *ca.* $83 \text{ g}(\text{m}^2)^{-1}$ peel is available.

Peel weight \times the actual number of J2(g peel) $^{-1}$ found after 240 days at storage temperature of 4°C, 8°C, and 12°C for cv. Desiree (Teklu *et al.*, 2018) and converted per g soil in m^2 , this would be 0.007, 0.022 and 0.204 J2(g soil) $^{-1}$, respectively. These P_i values are close to or higher than the quality thresholds reported by Ingham *et al.* (2000): *ca.* 0.004 J2 (g soil) $^{-1}$, Norshie *et al.* (2011): $< 0.5 \text{ J2 (g dry soil)}^{-1}$ and Teklu *et al.* (2016): over 4 experiments < 0.0625

J₂(g dry soil)⁻¹. Therefore, using susceptible potatoes as seed in an uninfected area poses a risk, both for infecting a new site as for direct quality damage of the produce. This risk can be avoided drastically when resistant genotypes *e.g.* ARo4-4096 are used with a $P_{\text{f}_{\text{tuber}}}$ of 0.002 J₂(g dry soil)⁻¹ after 300 days of storage at 7°C and *ca.* 0.0008 J₂(g dry soil)⁻¹ when stored at 4°C for 240 days.

Conclusion

Potato genotypes with resistance to *M. chitwoodi* follow a normal growth pattern. The introduction of these genotypes, when ready for practical use, will have no effect on the current potato growing conditions. Some genotypes showed yield reduction associated with delay at high nematode densities, which are not prevalent under normal growing conditions in spring. Tuber knot-index values for industrial processing are below rejection level, both in the four glasshouse experiments as in the two field experiments conducted so far (Teklu *et al.*, unpubl.). Thus, the use of these genotypes provides a direct solution for ware potatoes. Although the number of clean tubers of the genotypes is increased and tuber infestation is significantly lower than in tubers of susceptible cultivars, some tuber infestation occurs. It still has to be investigated how effective the resistance of these potato genotypes in preventing tuber infestation under field conditions, when population densities are kept at low levels using in integrated management approach.

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Chapter 4

Tuber and root resistance of potato genotypes against *Meloidogyne chitwoodi* in the presence of *Avena strigosa*, related to tuber quality

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Relative tuber infestation and quality of two *Meloidogyne chitwoodi* resistant potato genotypes, ARo4-4096 and 2011M1, were compared in glasshouse experiments at initial population density, $P_i = 16$ second-stage juveniles, J2(g dry soil)⁻¹ in the presence and absence of the bristle oat, *Avena strigosa*. When *A. strigosa* was added, $P_{\text{root+soil}}$ (P_f = final population) on both ARo4-4096 and 2011M1 increased 130×, $P_{\text{f tuber}}$ increased 1.9 and 3.7×, respectively, while $P_{\text{f tuber}} \times$ (FRW)⁻¹, fresh root weight, were the same. Nematode hatch from peel of ARo4-4096, without *A. strigosa*, was delayed by 3 weeks but relative maximum hatching rate was increased. Although the RS_{tuber} (RS = Relative Susceptibility) of both ARo4-4096 and 2011M1 were smaller than 1%, in the presence of *A. strigosa* tuber quality of 2011M1 dropped below the marketable level, while that of ARo4-4096 was hardly affected. We conclude that: **i)** $P_{\text{f tuber}}$ is influenced by root mass; **ii)** root quality (source of food) influences nematode hatch; **iii)** tuber quality is not an estimator for tuber resistance; and the reverse **iv)** root resistance is equal to tuber resistance.

Keywords: bristle oat, exogenous inoculum, relative host-status, resistance testing, root-knot nematode, tuber tolerance.

Introduction

Resistance genes against *Meloidogyne chitwoodi*, *M. hapla* and *M. fallax*, obtained from several wild tuber-bearing *Solanum* species (Janssen *et al.*, 1995, 1996; Brown *et al.*, 2006), have been used by Dutch potato breeders to develop potato genotypes with a single resistance gene against *M. chitwoodi* (Draaistra, 2006). Research by Norshie *et al.* (2011) and Teklu *et al.* (2016) proved that these genotypes are highly resistant and Teklu *et al.* (2016) distinguished two groups with different *M/a* ratios. The resistance of these genotypes, expressed as Relative Susceptibility (RS), estimated from the P_f in the roots and the soil was equal to the RS_{tubers} assessed after 240–300 days of storage. However, the RS_{tubers} estimates included only half of the genotypes and a comparison between the two *M/a* groups could not be made. According to Boydston *et al.* (2007), in the presence of hairy nightshade (*Solanum sarrachoides*), some tubers of the *M. chitwoodi* resistant genotype PA95B4-67 were infected, whereas tubers of genotype PA99N82-4 expressed tuber resistance, in both glasshouse and field trials. They estimated tuber resistance by counting the browned infection sites on the flesh of peeled tubers and rating them in five categories. Tubers with six or more brown spots were classified as culls. Brown *et al.* (2009), assessing tuber resistance in a similar way, distinguished two linked genetic factors that control resistance to *M. chitwoodi* race-1 in the roots and tubers of potato plants independently. These findings, combined with those of Teklu *et al.* (2016), raise the following questions:

- i) Is there a difference between root and tuber resistance?
- ii) Is the relative P_p estimated from the roots and soil of a resistant genotype, compared to that of a susceptible reference, a good estimator for both root and tuber resistance?
- iii) Is tuber quality, *e.g.*, categorised browned sites or proportions of culled tubers, a good predictor of tuber resistance?

To answer these questions, we designed a glasshouse test, similar to that of Boydston *et al.* (2007). Two newly developed resistant genotypes with different RS characteristics, expressed as different *M/a* ratios (Teklu *et al.*, 2016), AR04-4096 and 2011M1, were selected and grown in 10 kg pots. In half of these pots, a good host for *M. chitwoodi* was added. This host should satisfy one important criterion: it must not affect the growth and yield of the potato plants. After preliminary studies, the monocotyledon bristle oat, *Avena strigosa*, was chosen, instead of hairy nightshade as used by Boydston *et al.* (2007) and Brown *et al.* (2009). Potato cv. Desiree was used as a susceptible reference. There were a few adjustments to Boydston's and Brown's experimental design: the potato tubers and *A. strigosa* seeds were planted at the same time and inoculation with nematodes was done immediately before planting, to avoid biases in population dynamics.

Materials and methods

Experimental design

The experiment was conducted in glasshouses at two different locations of Wageningen University and Research, Wageningen (PRI) and Lelystad (PPO), using 10 kg pots, containing a soil mixture described by Teklu *et al.* (2014, 2016). Two genotypes, 2011M1 and ARo4-4096, were grown at PPO and PRI, respectively, with and without four plants of *A. strigosa* per pot. There were 10 replicates per object; cv. Desiree was used as susceptible reference and was grown without *A. strigosa* in five and two replicates at PRI and PPO, respectively.

Source of inoculum and nematode densities

Meloidogyne chitwoodi (Mc31 or the Smakt population) was multiplied on the susceptible tomato cv. Moneymaker for 3 months (Teklu *et al.*, 2016). Root systems were harvested and placed in a Seinhorst spray-mist chamber to collect second-stage juveniles (J2). Only freshly hatched J2, not older than 4 days, were used for inoculation. Plants were grown at P_i values of 16 J2(g dry soil)⁻¹. This P_i value was chosen so that P_f counts were sufficient to keep coefficients of variation below 10% and P_f was not affected by growth reduction (Teklu *et al.*, 2016).

Nematode inoculation

The J2 were inoculated into the soil, by inserting horse needles at 20 evenly spaced places in the pot, just prior to planting of the potato tubers and the *A. strigosa* seeds in order to mimic the natural processes in the field as far as possible, while controlling experimental variance (Been & Schomaker, 1986; Teklu *et al.*, 2016). The protocol at PPO was slightly modified by using a Jencons perimatic universal dispenser (Jencons Co.). During the inoculation process, the Poisson distribution of the nematodes in the suspensions was safeguarded and two samples were taken from the inoculum and counted to assess the density.

Planting and maintenance of plants

A cylindrical, 12 g, tuber piece with a single sprout was planted in the centre of each pot at a depth of 6 cm. At each location, in 10 of the 20 pots with potato genotypes, four seeds of *A. strigosa* were planted halfway between the tuber piece and the pot perimeter, at 2 cm depth and at equal distance from each other. The reference cv. Desiree was grown without *A. strigosa*. A perforated plastic sheet, cut 2-3 cm smaller than the diameter of the pot, was used to cover the top soil and to prevent both splashing and erosion during watering and excessive evaporation during the growing period. A more detailed description of this method can be found in Teklu *et al.* (2016). After planting, pots were positioned randomly

in the glasshouse. Temperatures were kept at 18-20°C during the day and at 16°C during the night, while 16 h of day light were maintained. Humidity was kept at 60-70%.

Before planting, soil moisture in the pots was raised to approximately 10% of the dry soil. After planting, moisture was maintained between 12 and 15%. Each week plant height was measured, pots were weighed and the required amount of water was added to compensate for evaporation and plant growth. Increase in plant weight was estimated from plant height. At the same time, pots were rotated within the glasshouse to avoid positional effect. In between weighing, when water use was presumed to be high, five randomly selected pots were weighed to assess water loss and an equal amount of water was added to all the pots.

During growth, extra fertiliser, NPK (12:10:18), was added. The plants at PPO received approximately 2.3× the normal total dosage of 1 g (kg soil)⁻¹ of NPK. Plants were grown for 16 weeks, which is the normal growing period of potatoes.

Observations and measurements

For convenience, object names were abbreviated. These abbreviations and a glossary of used variables and parameters are presented in Table 4.1.

Tuber quality

To clean tubers from adhering soil, they were gently washed and then dried at room temperature for 48 h. Next, the Tuber knot-index (TKI; Visser & Korthals, 2004), which is still used for industrial purposes, was estimated. Only tubers larger than 25 mm diameter in the middle of the longest side were scored. Tubers were allotted in five classes (Table 4.2). The TKI generates scores between 0 and 100. Maximum TKI scores of 10 or 20, depending on demand and supply, are accepted for industrial processing.

The main shortcoming of the TKI is that it does not represent a biological theory and as it is biased by human interpretation and multiplication rates per class are arbitrary, the results are variable and cannot be modelled. Moreover, as tubers with TKI = 0 still may have internal symptoms; it is not a suitable standard for seed potatoes. Therefore, also the proportions of symptomless tubers, F₀, (without internal or external symptoms) and the proportion of tubers in class 1, F₁, (with internal but without external symptoms) were estimated.

$$F_0 = \frac{cl_0}{\sum (cl_0 \dots cl_4)} \quad (1)$$

$$F_1 = \frac{cl_1}{\sum (cl_0 \dots cl_4)} \quad (2)$$

$$TKI = \frac{0 \times (cl_0 + cl_1) + 10 \times cl_2 + 33 \times cl_3 + 100 \times cl_4}{\sum (cl_0 \dots cl_4)} \quad (3)$$

cl_n = number of tubers in class n; $0 \leq n \leq 4$

Table 4.1. Glossary of terms used

Term	Description	Dimension
AR(-)	Potato genotype ARo4-4096 without <i>Avena strigosa</i>	-
AR(+)	Potato genotype ARo4-4096 with <i>A. strigosa</i>	-
M1(-)	Potato genotype 2011M1 without <i>A. strigosa</i>	-
M1(+)	Potato genotype 2011M1 with <i>A. strigosa</i>	-
Des(PRI-)	Potato cultivar Desiree (location PRI) without <i>A. strigosa</i>	-
Des(PPO-)	Potato cultivar Desiree (location PPO) without <i>A. strigosa</i>	-
FTW	Fresh Tuber Weight	g
FRW	Fresh Root Weight	g
FPW	Fresh Peel Weight	g
P_i	Nematode density at the time of planting	J2(g dry soil) ⁻¹
P_f	Nematode density at the time of harvest	J2(g dry soil) ⁻¹
F(t)	Logistic model	J2(g FPW) ⁻¹
t	Time	days
C or $P_{f\text{tub-gp}}$	maximum of F(t)	J2(g FPW) ⁻¹
A	Time when $0.5 \times C$ is reached	days
B	reciprocal of the relative rate of increase in F(t)	-
$P_{f\text{r+s}}$	Final population density in roots and soil	J2(g dry soil) ⁻¹
$P_{f\text{tub-gp}}$	Final population density in tubers	J2(g FPW) ⁻¹
$P_{f\text{tub-gs}}$	Final population density in tubers	J2(g dry soil) ⁻¹
$P_{f\text{tot}}$	$P_{f\text{r+s}} + P_{f\text{tub-gs}}$	J2(g dry soil) ⁻¹
TKI	Tuber knot-index	-
Fo	Proportion of tubers without internal or external symptoms	-
F1	Proportion of tubers with internal but without external symptoms	-
T	Tolerance limit: the maximum P_i value where Fo is estimated as 1	J2(g dry soil) ⁻¹
a	Maximum multiplication rate: $P_f \times P_i^{-1}$ at $P_i \rightarrow 0$	-
M	Maximum population density: P_f at $P_i \rightarrow \infty$	J2(g dry soil) ⁻¹
RS	Relative Susceptibility	%
RS _{r+s}	Percentage $P_{f\text{r+s}}$ on a genotype of the $P_{f\text{r+s}}$ on Desiree at $P_i = 16$	%
RSa _{r+s}	Percentage a_{r+s} on a genotype of the a_{r+s} on Desiree	%
RSM _{r+s}	Percentage M_{r+s} on a genotype of the M_{r+s} on Desiree	%
RS _{tub-gp}	Percentage $P_{f\text{tub-gp}}$ on a genotype of the $P_{f\text{tub-gp}}$ on Desiree at $P_i = 16$	%
RS _{tub-gs}	Percentage $P_{f\text{tub-gs}}$ on a genotype of the $P_{f\text{tub-gs}}$ on Desiree at $P_i = 16$	%
relFRW	$\text{FRW}_{\text{genotype}} \times \text{FRW}_{\text{Desiree}}^{-1}$	-

Table 4.2. Classification of tubers by rating external and internal symptoms, used for the estimation of the TKI, the tuber knot-index (Visser & Korthals, 2004).

Class	External symptoms	Internal symptoms
0	None	none
1	None	yes
2	on < 30% of tuber surface	yes
3	on 30-100% of tuber surface	yes
4	heavily deformed tubers	yes

Extraction of J2 from roots and soil (P_{r+s})

After removing the tubers, the soil was sieved through a 6 × 6 mm mesh to collect the root systems, which were then rinsed with water. After adhering water was allowed to drain for 20 min, Fresh Root Weights (FRW) were estimated. When *A. strigosa* was present, the joint FRW per pot were estimated.

The final population (P_{r+s}) was estimated by extracting and counting the nematodes from both the roots and the soil. Whole root systems were used to minimise variation. To extract the nematodes from the roots, they were cut into 1 cm pieces, placed in 9 cm diameter, 150 µm extraction sieves, on top of 12 cm diameter extraction dishes, and kept in a spray-mist chamber (Seinhorst, 1988; Teklu *et al.*, 2016) at 20°C for 4 weeks. Hatched J2 were collected at weekly intervals. To extract nematodes from the soil, a 500 g sub-sample of the well-mixed soil was elutriated using the Seinhorst elutriator (Seinhorst, 1962, 1988). Final nematode population (P_{r+s}) were estimated from the sum of nematodes extracted from the roots and the soil.

Extraction of nematodes from the potato tubers

Harvested tubers were exposed to 20°C for 2 weeks to harden their skin. All, whole tubers were peeled 5 mm deep (Viaene *et al.*, 2007). The peel was then cut into 1 cm² pieces, mixed thoroughly and – after the Fresh Peel Weights (FPW) were estimated – a 30 g sub-sample was collected to estimate number of J2 in the tubers. The samples were placed on 20 cm diameter, 425 µm extraction sieves, on top of 25 cm diameter extraction dishes. The samples were kept in a spray-mist chamber for 7 weeks, while hatched J2 were collected and counted every week (Teklu *et al.*, 2013, 2016).

Data analysis and Modelling

Data were analysed using scripts written with the Tinn-R editor version 4.0.2.1 and run in R version 3.2.2.

Plant weights

Averages and variances of FTW, FPW and FRW were estimated and the absolute value of differences, *D*, between relevant averages calculated. Equal variances, evaluated in variance

tests, were pooled to estimate the standard error of these differences, SEDav.

$$SEDav = \sqrt{SEav_1^2 + SEav_2^2} \quad (4)$$

The parameter SE is the standard error of the two averages, av_1 and av_2 . Then, probabilities (P) in R language) of $D = 0$ were estimated in two sample t -tests (Dalgaard, 2002). The variable D was considered to be larger than zero when $P < 0.05$. When more than two means were compared, one-way ANOVA was done, combined with pairwise t -tests with *holm* corrections of the P -values for multiple testing (Dalgaard, 2002).

Final population densities from roots and soil

The average and variance of the final population densities from roots and soil ($P_{f_{r+s}}$) was estimated in a similar manner as the plant weights, and statistical procedures were the same. The only difference was that these data were first log-transformed. Because of these log transformations, the SED of two log-transformed averages is defined as

$$SEDlav = \sqrt{\log(CVlav_1^2 + CVlav_2^2) + 1} \quad (5)$$

CV is the coefficient of variation and lav_1 and lav_2 are two log-transformed averages (Mood *et al.*, 1986).

Final population densities from the tubers ($P_{f_{tuber}}$)

In the final population densities from the tubers ($P_{f_{tub-gp}}$) from resistant genotypes zeros occurred. These zeros were considered small numbers and replaced by $0.5 \times$ the minimum (hMin) within a set of replicates. The minimum of hMin was 0.0005. When all replicates of $P_{f_{tub-gp}}$ were zero, the averages were considered 'true' zeros. In the regression analysis, these 'true' zeros were replaced by a very small number, 1×10^{-7} , that neither affected the parameter estimates (all larger than 1) nor their standard errors until the seventh decimal. After log transformation, averages were calculated and a logistic model (Equation 6) was fitted to the data and the three parameters were estimated. Standard errors of the parameters were estimated from the inverse of the Hessian Matrix (Venables *et al.*, 2016).

$$F(t) = \frac{C}{1 + \exp((A - t) / B)} \quad (6)$$

Where:

- t hatching time (day)
- C maximum cumulative hatch ($J_2(g \text{ FPW})^{-1}$)
- B the reciprocal of the relative rate of increase
- A time when $F(t) = 0.5 \times C$ (day)

Absolute differences between the same parameters values of relevant treatments were compared with their Least Significant Differences (LSD), calculated from pooled standard errors and 0.975 quantiles of the Student's t -distribution, for the given degrees of freedom. To compare estimates of parameter C ($P_{f_{tub-gp}}$), these values were log transformed and

SEDlav of $P_{f_{\text{tub-gp}}}$ was estimated according to Equation 5. For the statistical tests on *A* and *B*, Equation 4 was used. To enable comparison of the P_f from the tuber fraction with the P_f from the root and soil fraction, $P_{f_{\text{r+s}}}$, the dimensions of both variables must be made the same. Therefore, P_f tuber per g peel weight, $P_{f_{\text{tub-gp}}}$, was expressed as P_f tuber per g soil, $P_{f_{\text{tub-gs}}}$, using FPW of whole tubers per pot. In addition, the total P_f per g soil, $P_{f_{\text{tot}}}$, was calculated, adding $P_{f_{\text{tub-gs}}}$ and $P_{f_{\text{r+s}}}$.

In search for similarities, rather than differences, ratios of $P_{f_{\text{tub-gp}}}$ and FRW on the same genotype, with and without *A. strigosa*, were calculated.

Relative susceptibilities

For AR(-) both $RS_{f_{\text{r+s}}}$ and $RS_{f_{\text{tub-gp}}}$ were calculated. As we have, only one P_i value, RS is here estimated as

$$RS = \frac{P_{f_{\text{res}}}}{P_{f_{\text{sus}}}} \quad (7)$$

Where $P_{f_{\text{res}}}$ and $P_{f_{\text{sus}}}$ are the P_f estimates on a resistant genotype and cv. Desiree, respectively. The $RS_{f_{\text{tub-gp}}}$ adjusted for FRW, $RS_{f_{\text{tub-gp}}} \times \text{relFRW}^{-1}$, is estimated from Equation 7, with the P_f values divided by the corresponding FRW's:

$$RS_{f_{\text{tub-gp}}} \times \text{relFRW}^{-1} = \frac{P_{f_{\text{res}}}}{\text{FRW}_{\text{res}}} : \frac{P_{f_{\text{sus}}}}{\text{FRW}_{\text{sus}}} = \frac{P_{f_{\text{res}}}}{\text{FRW}_{\text{res}}} \times \frac{\text{FRW}_{\text{sus}}}{P_{f_{\text{sus}}}} = \frac{P_{f_{\text{res}}}}{P_{f_{\text{sus}}}} \times \frac{\text{FRW}_{\text{sus}}}{\text{FRW}_{\text{res}}} \quad (8)$$

Where

$$\text{relFRW} = \frac{\text{FRW}_{\text{res}}}{\text{FRW}_{\text{sus}}} \quad (9)$$

Again, the subscripts 'res' and 'sus' refer to one of the resistant genotypes and the susceptible reference, cv. Desiree, respectively.

Results

Plant weights

One of the two replicates of Des(PPO-) showed poor growth. Therefore, these data were excluded from data analysis.

FRW of AR(-), M1(-) and Des(PRI-) were the same: 14 g on average. The FRW of Des(PPO-) was 52.9 g, 3.7× higher. The FRWs of AR(+) and M1(+) were 1.8 and 3.4× as large as that of AR(-) and M1(-), respectively, implying that the average FRW of *A. strigosa* were 37.2 and 9.6 g at PPO and PRI locations, respectively. The presence of *A. strigosa* did not affect FTW. Due to a different fertilisation regime, FTW were higher at PPO than at PRI, although the FTW of Des(PPO-) was only 55% of the FTW of M1(-) and M1(+), also grown at the PPO location. More details of FRW and FTW are presented in Table 4.3.

Table 4.3. Plant weights of genotypes without (-) and with (+) *A. strigosa*. Lab = laboratory; N = number of replicates; FRW = g Fresh Root Weight; FTW = g Fresh Tuber Weight; FPW = g Fresh Peel Weight; M1(+) and AR(+) = 2011M1 and AR04-4096 with *A. strigosa*; M1(-) and Des(PPO-), = 2011M1 and Desiree at PPO without *A. strigosa*; AR(-) and Des(PRI-) = AR04-4096 and Desiree at PRI without *A. strigosa*. Equal variables are marked with the same letter.

Genotype (+) or (-)	Lab	N	FRW	FTW	FPW
<i>A. strigosa</i>					
M1(+)	PPO	10	52.9 ^a	580.6 ^a	183.5 ^a
M1(-)	PPO	10	15.7 ^b	498.6 ^a	180.5 ^a
Des(PPO-)	PPO	1	52.9 ⁻	296.0 ⁻	132.1 ⁻
AR(+)	PRI	10	21.6 ^c	184.0 ^b	72.0 ^b
AR(-)	PRI	10	12.0 ^b	195.1 ^b	72.1 ^b
Des(PRI-)	PRI	5	14.0 ^b	132.0 ^c	57.8 ^c

Final population densities

Final population from roots and soil ($P_{f_{root+soil}}$)

All estimates of final population densities, P_p , are summarized in Table 4.4.

Table 4.4. Final population densities of *Meloidogyne chitwoodi*, P_p in roots and soil and the tubers of genotypes without (-) and with (+) *A. strigosa* and cv. Desiree. Lab = Laboratory, N = number of replicates; FRW=g Fresh Root Weight; $P_{f_{r+s}} = P_f$ in roots and soil, $J_2(g \text{ dry soil})^{-1}$; $P_{f_{tub-gp}} = P_f$ in tubers, $J_2(g \text{ peel})^{-1}$; $P_{f_{tub-gs}} = P_f$ in tubers, $J_2(g \text{ dry soil})^{-1}$; $P_{f_{tot-gs}} = \text{sum of } P_{f_{r+s}} \text{ and } P_{f_{tub-gs}}$, $J_2(g \text{ dry soil})^{-1}$; Ratio (+/-) = ratio of P_f values on a given genotype with and without *A. strigosa*. Equal variables are marked with the same letter.

Genotype (+) or (-)	Lab	N	$P_{f_{r+s}}$	Ratio (+/-) $\frac{P_{f_{tub-gp}}}{P_{f_{r+s}}}$	$P_{f_{tub-gp}}$	Ratio (+/-) $\frac{P_{f_{tub-gp}}}{P_{f_{tub-gp}}}$	$P_{f_{tub-gp}}$ $\times FRW^{-1}$	Ratio (+/-) $\frac{P_{f_{tub-gp}}}{P_{f_{tub-gp}} \times FRW^{-1}}$	$P_{f_{tot-gs}}$	$\%P_{f_{tub-gs}} \times P_{f_{tot-gs}}$
<i>A. strigosa</i>										
M1(+)	PPO	10	30.53	x 127 ^a	37.7 ^a	x 3.7 ^a	0.71 ^a	x1.1 ^a	31.2	2.2
M1(-)	PPO	10	0.24		10.2 ^b		0.65 ^a		0.4	43.5
Des(PPO-)	PPO	1	91.29		3641.6		70.03		139.4	34.5
AR(+)	PRI	10	9.09	x 133 ^a	2.4 ^c	x 1.9 ^b	0.11 ^b	x1.0 ^a	9.1	0.2
AR(-)	PRI	10	0.07		1.3 ^d		0.11 ^b		0.1	12.0
Des(PRI-)	PRI	5	41.41		728.6 ^e		52.04		45.6	9.2

Although the final population density from roots and soil, $P_{f_{r+s}}$, was 3.5× higher on M1(-) than on AR(-), both numbers were increased by the same amount, on average 130×, on the combined root systems of either genotype and *A. strigosa*. The $P_{f_{r+s}}$ on Des(PRI-) was 41.4 $J_2(g \text{ dry soil})^{-1}$, 4.6× higher than on AR(+), while $P_{f_{r+s}}$ on the one replicate of Des(PPO-) was 91.3, three times higher than on M1(+).

Final population from the tuber peel (P_{tuber})

The parameter estimates, C , A and B of the logistic model and their coefficients of variation (CV) are presented in Table 4.5. For hatched J2 from peel of AR(-) both parameters B^{-1} (the relative rate of increase) and A were larger than for the other objects. In Figure 4.1 the hatching curves of J2 from tuber peel of AR(-), AR(+) are compared with those of M1(-) and M1(+). Most notable is the delay of 21 days in hatching of nematodes from peel of AR(-), followed by a 2.7× higher relative rate of increase, B^{-1} . The final population, $P_{\text{tuber}} \text{ (g FPW)}^{-1}$, $P_{\text{tub-gp}}$, was estimated by the parameter C . The $P_{\text{tub-gp}}$ of M1(-) was 7.8× higher than $P_{\text{tub-gp}}$ of AR(-).

Table 4.5. Parameter estimates of *Meloidogyne chitwoodi* hatching from potato genotypes without (-) and with (+) *A. strigosa* and cv. Desiree and their coefficients of variation (CV) of the logistic model $F(t) = \frac{C}{1 + \exp((A-t)/B)}$ (Equation 6). Lab = Laboratory, N = number of replicates; C = maximum cumulative hatch per gram peel weight ($P_{\text{tub-gp}}$); B = the relative rate of increase; A = time (in days) when $0.5 \times C$ is reached; t is hatching time in days. Equal variables are marked with the same letter.

Genotype (+) or (-) <i>A. strigosa</i>	Lab	N	$C (P_{\text{tub-gp}})$	B	A	CV_C	CV_B	CV_A	R^2
M1(+)	PPO	10	37.7 ^a	6.5 ^a	24.0 ^a	0.03	0.08	0.03	0.99
M1(-)	PPO	10	10.2 ^b	8.6 ^a	22.4 ^a	0.10	0.21	0.08	0.98
Des(PPO-)	PPO	1	3641.5	5.9	22.7	0.04	0.14	0.04	0.99
AR(+)	PRI	10	2.4 ^c	6.9 ^a	29.9 ^a	0.07	0.21	0.09	0.98
AR(-)	PRI	10	1.3 ^d	2.8 ^b	37.9 ^b	0.01	0.07	0.02	0.99
Des(PRI-)	PRI	5	728.6 ^e	9.6 ^a	26.6 ^a	0.07	0.13	0.06	0.99

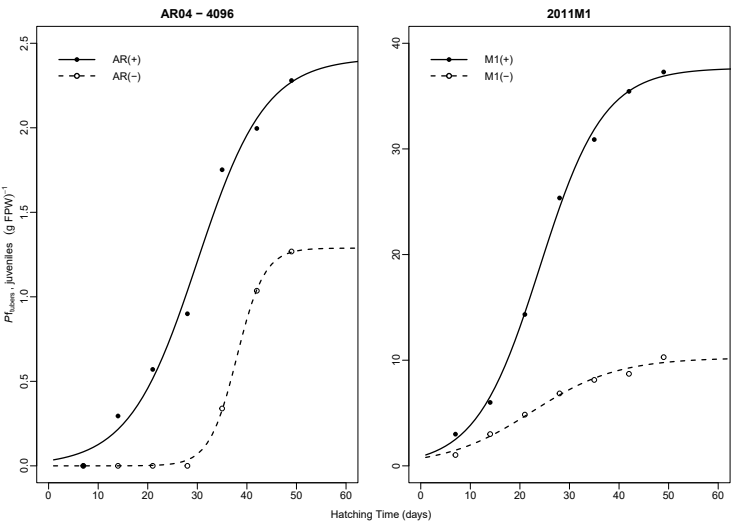


Figure 4.1. Cumulative hatching curves of second-stage juveniles (J2) of *Meloidogyne chitwoodi* from tuber peel ($P_{\text{tub-gp}}$) of potato genotypes AR04-4096 and 2011M1, both without (dotted line) and with *Avena strigosa* (solid line). See Table 4.1 for definition of terms. The logistic model $F(t) = \frac{C}{1 + \exp((A-t)/B)}$ (Equation 6) was fitted to the data.

In the presence of *A. strigosa*, the $P_{f_{\text{tub-gp}}}$ on both 2011M1 and ARo4-4096 were increased at slightly different rates: 3.7 and 1.9, respectively. However, $P_{f_{\text{tub-gp}}} \times \text{FRW}^{-1}$ was the same for each genotype pair: 0.68 for M1(+) and M1(-) and 0.11 for AR(+) and AR(-).

Total final population density ($P_{f_{\text{total}}}$)

The total P_f $P_{f_{\text{tot-gs}}}$, expressed as $\text{J}_2(\text{g dry soil})^{-1}$, was estimated by adding $P_{f_{r+s}}$ and $P_{f_{\text{tub-gs}}}$. The percentage of $P_{f_{\text{tot-gs}}}$ originating from the tubers ($P_{f_{\text{tub-gs}}}$) was then estimated to be 43.5 and 34.5 for M1(-) and Des(PPO-), and 12 and 9.2 for AR(-) and Des(PRI-), respectively. In the presence of *A. strigosa* the $P_{f_{\text{tub-gs}}}$ estimates, as percentages of $P_{f_{\text{tot-gs}}}$, were much smaller: 2.2% and 0.2% on M1(+) and AR(+), respectively.

Relative susceptibilities

Table 4.6 shows the estimates of RS_{r+s} , RSM_{r+s} , $\text{RS}_{\text{tub-gp}}$ and $\text{RS}_{\text{tub-gp}} \times \text{relFRW}^{-1}$ in comparison with the tuber quality estimates, Fo, F1 and TKI.

The RS_{r+s} of AR(-) and M1(-) at $P_i = 16 \text{ J}_2(\text{g dry soil})^{-1}$ were estimated to be 0.17% and 0.26%, respectively. As the M/a ratios on ARo4-4096 and on cv. Desiree are the same: 1.3 (Teklu *et al.*, 2016), this RS_{r+s} estimate for AR(-) is P_i independent and is equal to both $\text{RS}_{a_{r+s}}$ and RSM_{r+s} . Contrary to ARo4-4096, the M/a ratio on 2011M1 is 8.6 and 6.6× higher than on cv. Desiree. From the data of Teklu *et al.* (2016), we learn that $P_{f_{r+s}}$ on M1(-) is $0.65 \times M$ at $P_i = 16 \text{ J}_2(\text{g dry soil})^{-1}$, whilst $P_{f_{r+s}}$ on cv. Desiree at $P_i = 16 \text{ J}_2(\text{g dry soil})^{-1}$ equals $0.92 \times M$.

Table 4.6. Relative Susceptibilities (%RS) to *Meloidogyne chitwoodi* of genotypes without (-) and (+) with *A. strigosa*, compared to their tuber quality estimates Fo, F1 and TKI. Lab = Laboratory; N = number of replicates; FPW = g Fresh Peel Weight; FRW = g Fresh Root Weight; $P_{f_{r+s}} = P_f$ in the roots and the soil, $\text{J}_2(\text{g dry soil})^{-1}$; $P_{f_{\text{tub-gp}}} = P_f$ in the tubers, $\text{J}_2(\text{g peel})^{-1}$; $\text{RS} = 100 \times P_{f_{\text{genotype}}} \times P_{f_{\text{Desiree}}}^{-1}$ at $P_i = 16 \text{ J}_2(\text{g dry soil})^{-1}$; $\text{RS}_{r+s} = \% \text{RS}$ estimated from $P_{f_{r+s}}$; $\text{RS}_{\text{tub-gp}} = \% \text{RS}$ estimated from $P_{f_{\text{tub-gp}}}$; $\text{RSM}_{r+s} = \% \text{RS}$ estimated from the parameters M ; M = the maximum P_f at $P_i \rightarrow \infty$; $\text{relFRW} = \text{FRW}_{\text{genotype}} \times \text{FRW}_{\text{Desiree}}^{-1}$; Fo = the proportion of tubers without external or internal symptoms; F1 = the proportion of tubers without external symptoms but with internal symptoms; TKI = Tuber knot-index; $\text{hs} = 100 \times P_{f_{r+s}} (+) \times P_{f_{r+s} \text{ Desiree}}^{-1}$. NA = missing value. Equal variables are marked with the same letter.

Genotype (+) or (-)	Lab	N	RS_{r+s} $P_i = 16$	RSM_{r+s}	$\text{RS}_{\text{tub-gp}}$ $P_i = 16$	$\text{RS}_{\text{tub-gp}} \times \text{relFRW}^{-1}$	Fo	F1	TKI
<i>A. strigosa</i>									
M1(+)	PPO	10	^{hs} 33.45	NA	1.03	1.02 ^a	0.19 ^a	0.04 ^a	28.1
M1(-)	PPO	10	0.26	0.38	0.28	0.93 ^a	0.70 ^b	0.06 ^a	4.0
Des(PPO-)	PPO	1	100	100.00	100.00	100.00	0.00 ⁻	0.00 ^b	100.0
AR(+)	PRI	10	^{hs} 21.96	NA	0.33	0.21 ^b	0.82 ^c	0.05 ^a	1.3
AR(-)	PRI	10	0.17	0.17	0.18	0.21 ^b	0.93 ^c	0.05 ^a	0.2
Des(PRI-)	PRI	5	100.00	100.00	100.00	100.00	0.19 ^a	0.00 ^b	66.7

To make an unbiased comparison between the two genotypes, RS_{r+s} of M1(-) was multiplied by 0.92×0.65^{-1} to obtain RSM_{r+s} : 0.38%. The estimates of the host status of M1(+) and AR(+), compared to Des(PPO-) and Des(PRI-), were 33.5% and 22.0%, respectively. While, RS_{tub-gp} of AR(-) and M1(-) were estimated to be 0.18% and 0.28% respectively.

The RS_{tub-gp} estimates of AR(-) and M1(-) were equal to their RS_{r+s} . Because of the one useable replicate of Des(PPO-), this could only be proven statistically for M1(-). The Relative Susceptibility, RS_{tub-gp} of M1(+), compared to Des(PPO-) was 1.03%, while the RS_{tub-gp} of AR(+), compared to Des(PRI-) was 0.33%. However, $RS_{tub-gp} \times relFRW^{-1}$, based on the $P_{fub-gp} \times gFRW^{-1}$ estimates (Equation 8) were the same within the genotype pairs: 0.97% for M1(+) and M1(-) and 0.21% for AR(+) and AR(-).

Tuber quality

F0 and F1, proportions of symptomless and external symptomless tubers

At both locations, the average numbers of tubers on the resistant genotypes were the same, 9.5 tubers pot^{-1} , and independent of the presence of *A. strigosa*. The proportion of symptom-free tubers, Fo, of AR(-) and of AR(+) were the same: on average 0.87. For M1(-), the estimate of Fo was 0.7 slightly smaller than for AR(-), but Fo of M1(+) decreased to 0.19 and was equal to the Fo of Des(PRI-). When we evaluate the tubers only by their external symptoms by estimating F1, a 4 - 6% increase of marketable tubers is gained for both genotypes, irrespective of the presence of *A. strigosa*. For Des(PRI-) and Des(PPO-) no difference between Fo and F1 was observed. Details are summarised in Table 4.6.

Tuber knot-index

The TKI of AR(-) and M1(-) were 0.2 and 4.0, respectively and increased to 1.3 and 28.1 in the presence of *A. strigosa*. The TKI of the susceptible Des(PRI-) and Des(PPO-) were estimated to be 66.7 and 100, respectively. More details are given in Table 4.6.

Discussion

Apart from the two genotypes and the absence or presence of *A. strigosa*, location was an additional source of variation in this experiment. For instance, the FRW of *A. strigosa* in M1(+), FTW and all the P_f estimates were higher at PPO than at PRI. Therefore, without making compensations for these differences, an unbiased comparison between AR04-4096 and 2011M1 is impossible. Without compensations, it would seem that FTW of 2011M1 is much higher than that of AR04-4096. However, if FTW at PPO would have been the same as at PRI, the FTW of Des(PPO-) would have been 132 g instead of 296 g and FTW of M1(+) and M1(-) would have been 259 and 222 g, about $1.3 \times$ the average FTW of AR(+) and AR(-). Reversed, the FRW of Des(PRI-), M1(+) and M1(-), if grown at PRI, would have been proportionally smaller. Despite the increased P_f values at PPO, both root and tuber

resistance could be estimated and unbiased comparisons made by calculating the Relative Susceptibilities, RS_{r+s} and RS_{tub-gp} , respectively, taking P_{f+s} and $P_{f-tub-gp}$ of Des(PPO-) and Des(PRI-) as susceptible references.

Final population densities and relative susceptibilities

$P_{f_{root+soil}}$ and $RS_{root+soil}$

Visser & Molendijk (2015) consider *A. strigosa* to be a good host for *M. chitwoodi*, but the multiplication rates in this experiment at $P_i = 16$ were small: 1.9 and 0.56 for M1(+) and AR(+), respectively. Its relative host status, hs (Table 4.6), under the given conditions, compared to cv. Desiree at $P_i = 16$ J2(g dry soil)⁻¹, is also modest: 22% at PRI in the presence of AR04-4096, and 33% at PPO in the presence of 2011M1. As we have neither estimates of a and M of *M. chitwoodi* on *A. strigosa*, nor of M/a ratios, extrapolating these results to lower or higher P_i values than 16 J2(g dry soil)⁻¹ is not possible.

Although FRW of *A. strigosa* was about 37 g at PPO, 3.7× higher than at PRI, P_{f+s} was increased at the same rate, 130×, on both locations when four *A. strigosa* plants were added to the resistant potato genotypes. Phrased in mathematics:

$$(P'_{f1} + P_{f2}) \times P_{f1}^{-1} = P'_{f1} \times P_{f1}^{-1} + P_{f2} \times P_{f1}^{-1} = c + P_{f2} \times P_{f1}^{-1} = 130 \quad (10)$$

Where P'_{f1} is P_{f+s} of either genotype in the presence of *A. strigosa*; P_{f1} is P_{f+s} of the genotypes without *A. strigosa*; and P_{f2} is P_{f+s} on *A. strigosa* in the presence of a resistant potato genotype. Should $P'_{f1} = P_{f1}$, then $c = 1$. If $P'_{f1} < P_{f1}$ then $c < 1$. In the unlikely event that $P'_{f1} > P_{f1}$, say $P'_{f1} = 2 \times P_{f1}$, then $c = 2$. We conclude that, as long as c is small compared to 130, Equation 7 equals close to $P_{f2} \times P_{f1}^{-1}$. Therefore, it is safe to conclude that the extra roots of *A. strigosa* in pots of M1(+), compared to AR(+), did not affect P_{f+s} .

Relative susceptibilities were estimated to compare the resistance of the potato genotypes at the two locations, using the respective Des(PPO-) and Des(PRI-) as susceptible references. Relative P_f estimates at one P_i value, as calculated by e.g. Ferris *et al.* (1993) need not be the same at all P_i values, as $P_i \sim P_f$ lines of tested potato cultivars do not always run parallel with their susceptible references (Seinhorst *et al.*, 1995; Teklu *et al.*, 2016). We know from earlier experiments that M/a ratios of 2011M1 on the one hand and cv. Desiree and eight other resistant genotypes, among them AR04-4096, on the other hand, are different: 1.3 for cv. Desiree and AR04-4096 and 8.6 for 2011M1. Only with this knowledge is it possible to extrapolate relative P_f values, estimated at a given P_i , here $P_i = 16$ J2(g dry soil)⁻¹, to other P_i values.

$P_{f_{tuber}}$ and RS_{tuber}

Final population from tubers, $P_{f-tub-gp}$ J2(g FPW)⁻¹, of both genotypes, grown in the presence of *A. strigosa*, increased at a much smaller rate than P_{f+s} : 3.7 and 1.9 for M1(+) and AR(+). Therefore, it is probable that $P_{f-tub-gp}$ of either genotype reached its maximum under the given conditions. As the $P_{f-tub-gp} \times FRW^{-1}$ estimates within the genotype pairs AR(+), AR(-)

and $M_1(+)$ and $M_1(-)$ were the same, the $RS_{\text{tub-gp}} \times \text{relFRW}^{-1}$ are also the same, irrespective of the estimates of $P_{\text{f-tub-gp}}$ or FRW for cv. Desiree at the two locations.

Root quality influencing $P_{\text{f-tub-gp}}$

As only one mist chamber was used for the extraction of the nematodes from the peel, all the hatching data could be compared. We found significant differences: increase in the parameters A and B^{-1} in AR(-) compared to AR(+), $M_1(-)$, $M_1(+)$ and Des(-), implying an initial delay of the hatching process of nematodes from AR(-) peel and a speedier hatching later on. There are two possibilities for causing the delay:

- i) A toxic component from which the nematodes have to recover. This was observed earlier by Schomaker & Been (1999) in nematodes treated with metam-sodium or 1, 3-dichloropropene. Their explanation of this phenomenon was that the nematodes had been exposed to sub lethal dosages and needed time to recover. In this experiment, this toxic component could only come from the tubers.
- ii) Stagnation in development of J2 in the AR(-) tubers. Hypothesis i) cannot be true as the nematodes from the peel of AR(+) hatched normally. Thus, the most likely cause of the parameter increase of AR(-) is that the hatchability of nematodes from the peel was determined by the quality of the root systems, from which the tuber-invading J2 originated. The majority of the nematodes from the peel of AR(+) had parents that developed on the roots of *A. strigosa*, whilst the nematodes from the peel of AR(-) descended from J2 developed on roots of AR04-4096. Compared to AR(-) roots, other roots, including $M_1(-)$, are superior as a food source for *M. chitwoodi* and as a consequence the J2 from these roots were in a better condition when they invaded the tubers. This theory is supported by Teklu *et al.* (2016), who found RSM of $M_1(-)$ to be ten times as high as that of seven other resistant genotypes. Most probably the offspring of AR(-) tuber-invading J2 could not finish their life cycle and it took them 3 weeks to do so in the mist chamber. At the end of this period, they reached the J2 stage at almost the same time, producing a steep hatching curve with a large relative rate of increase, B^{-1} .

Root quantity influencing $P_{\text{f-tub-gp}}$

Although the $P_{\text{f-tub-gp}}$ on AR(+) and $M_1(+)$ were increased slightly, the presence of *A. strigosa* did not influence the $P_{\text{f-tub-gp}} \times (\text{g FRW})^{-1}$. As this variable is the same within both the pairs AR(-), AR(+) and $M_1(-)$, $M_1(+)$, we conclude that the host status of the roots does not affect $P_{\text{f-tub-gp}}$, probably because the maximum of $P_{\text{f-tub-gp}}$ is reached and there is a surplus of J2 that can invade the tubers. Of course, this maximum depends on the degree of resistance, hence the difference between 2011M1 and AR04-4096. The estimates of $RS_{\text{tub-gp}} \times \text{relFRW}^{-1}$, the RS of tubers based on $P_{\text{f-tub-gp}} \times \text{FRW}^{-1}$, were the same within genotypes. For AR04-4096, $RS_{\text{tub-gp}} \times \text{relFRW}^{-1}$ estimates are equal to $RS_{\text{tub-gp}}$ and $RS_{\text{r+s}}$. Because of the unusual high

FRW of Des(PPO-), compared to M1(-), this is not true for 2011M1 but $RS_{\text{tub-gp}}$, based on $P_{\text{f-tub-gp}} \times \text{FRW}^{-1}$, and $RS_{\text{r+s}}$ would have been the same if FRW of cv. Desiree had equalled that of M1(-) as was true in most of our experiments (Teklu *et al.*, 2016). As there is only one replicate, we cannot be sure about the true estimates of Des(PPO-) variables and, therefore, not of the true RS of M1(-) and M1(+). Nevertheless, we are certain that $P_{\text{f-tub-gp}} \times \text{FRW}^{-1}$ estimates, and RS estimates derived thereof, are the same for M1(+) and M1(-) as they are for AR(-) and AR(+). Thus, the conclusion that only total FRW matters and not the host status of these roots, as long as they are hosts, is justified. Our findings, that it is the root quantity and at some level, root quality, that determine $P_{\text{f-tub-gp}}$, explain why inoculating resistant potato plants with J2 at the time of tuber setting, without increasing FRW, does not affect tuber quality (Mojtahedi *et al.*, 1995).

Tolerance for reduction of tuber quality

Although the presence of *A. strigosa* increased $P_{\text{f-tub-gp}}$ on AR(+) and M1(+) only slightly and $RS_{\text{tub-gp}}$ was always $\leq 1\%$, tuber quality of AR04-4096 was hardly affected, while for M1(+), the proportion of symptomless potatoes, Fo, dropped to 0.19 and TKI was raised above marketable levels. Obviously, in the presence of hosts, irrespective of the host status, tuber quality can be greatly affected. We do not know if and how the unusual *M/a* ratio of 2011M1 of 8.6 (Teklu *et al.*, 2016) influences this phenomenon. Quality tests where potatoes are grown in the presence of hosts, not affecting potato growth, may be a preliminary method to detect these genotypes. To predict future results, a better understanding of the underlying biological processes is necessary. This is only possible with additional data, allowing more extended modelling.

For M1(-), AR(+) and AR(-) the $RS_{\text{tub-gp}}$ estimates are accompanied by high values of Fo: 0.70, 0.82 and 0.93, respectively. By contrast, for M1(+), a 0.75% increase in $RS_{\text{tub-gp}}$ results in a decrease of almost 50% in Fo, while TKI estimates were raised above the levels of 10 or 20, required for industrial processing. One could argue that in spring P_i -values will be $10 \text{ J2(g dry soil)}^{-1}$ at a maximum and that these quality losses will hardly occur in practice. However, P_i -Fo relationships in earlier experiments demonstrate that the tolerance limits for Fo (T , the maximum P_i value where Fo is estimated to be 1) were $0.1 < T < 100$ for 2011M1 and $10 < T < 128$ for AR04-4096 (Teklu *et al.*, unpubl.). This is not the first time that we observe lack of correlation between resistance and tolerance (Trudgill, 1991). This is true for most nematode plant combinations. The $RS_{\text{r+s}}$ and $RS_{\text{tub-gp}}$, root and tuber resistance, of the genotypes without *A. strigosa* were the same: 0.27% for M1(-) and 0.18% for AR(-). For AR(-) the $RS_{\text{r+s}}$ estimates are, on average, in agreement with Teklu *et al.* (2016). For 2011M1 we cannot make these comparisons.

Final considerations

Our research did not provide evidence for a difference between root and tuber resistance. Tuber tolerance is not a good estimator for tuber resistance, nor are RS_{tuber} or $RS_{\text{r+s}}$ indicators of tolerance. Hence, we need separate tests for resistance and tolerance. The relative P_p estimated from the roots and soil of a resistant genotype, compared to that of a susceptible reference, can be a good estimator for both root and tuber resistance, as long as the M/a ratios of the test and reference cultivars are the same (Teklu *et al.*, 2016). The role of M/a ratios in these issues is still uncertain.

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Chapter 5

The effect of storage time and temperature on the population dynamics and vitality of *Meloidogyne chitwoodi* in potato tubers

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The population densities of *Meloidogyne chitwoodi* in potato tubers stored at 4°C, 8°C and 12°C after 0, 60, 120, 180 and 240 days of storage were assessed. Compared to control day = 0, storage temperatures of 4°C and 8°C reduced population densities to 9% and 35% after 240 days of storage, respectively, while nematode numbers in tubers at 12°C increased by 2.5 times. The maximum relative hatching rate of nematodes (B) from tubers stored at 8 and 12°C, increased linearly with storage time. At 4°C it remained constant. The time required ($2 \times A$) for the hatching process to reach the maximum number of juveniles (J_2) decreased with increasing storage temperature. Recovered J_2 of *M. chitwoodi* from tubers after 180 and 240 days of storage, at all three temperatures, proved to be still infective and able to multiply on cv. Desiree with estimates of the maximum multiplication rate (a) and the maximum population density (M) of 63.6 and 70.8 $J_2(\text{g dry soil})^{-1}$, respectively.

Keywords: hatching curves, infectivity, tuber peel, tuber storage, viability

Introduction

The root-knot nematodes, *Meloidogyne chitwoodi* (Golden *et al.*, 1980) and *M. fallax* (Karssen, 1996), are present on the EU quarantine pest list (Anonymous, 2000) as well as on the 'EPPO A2 List of pests recommended for regulation as quarantine pests' (EPPO/ OEPP, 2014). The presence of these two nematodes results in severe quality loss of below ground produce, *e.g.* carrots, black salsify and potato tubers. On potato tubers heavy blistering of the tuber skin and round brown to black spots when peeled, are known symptoms (Norshie *et al.*, 2011; Teklu *et al.*, 2016a).

One of the major means of dispersal of *M. chitwoodi* is infected seed *e.g.* potato tubers, bulbs, etc. (MacLeod *et al.*, 2012). According to EU legislation, seed potatoes have to be cultivated on pest-free areas to prevent further spread. Prior to export or marketing, the potatoes are checked for symptoms of *M. chitwoodi* (EPPO/OEPP, 2013). In the case of detection, specific phytosanitary actions have to be taken by Plant Protection Services to prevent further dispersal, which include rejecting the consignment for seed (EPPO/OEPP, 2006).

After harvest, potatoes are often stored until they are shipped. Storage can occur under different temperatures regimes based on the purpose of the potato tubers. For example seed potatoes are stored at 3–4°C (Struik & Wiersema, 2012) and ware potatoes for French fries are stored at 7–9°C. A low sugar level is required for the chips industry to prevent dark colouring of fried chips and a storage temperature of 8–10°C (Van der Zaag, 1992) is recommended. A relative humidity of up to 95% with adequate ventilation is recommended for prolonged storage (Van der Zaag, 1992). At all storage temperatures the potato is biologically active, *e.g.* respiration, sprouting, rotting and sweetening occurs. Selection of the correct storage temperature reduces post-harvest losses. On the other hand, storage conditions can also influence the population dynamics of pests present in the tubers. It is generally believed that population densities of root-knot nematodes, *e.g.* *M. chitwoodi* and *M. incognita*, continue to develop during storage and can reduce the quality of the produce (Jatala *et al.*, 1982; Ingham *et al.*, 2000; Powers *et al.*, 2005; Strand, 2006). Remarkably, none of the cited references or any others that can be found in the literature provides any quantitative data.

As a quarantine pest, the topic of prevention of spread of *M. chitwoodi*, especially by means of infested seed potatoes, becomes more important. The primary objective of this research was therefore to assess the population dynamics of *M. chitwoodi* in time under different storage temperatures. The selected storage temperatures used in this experiment were based on storage temperatures currently used for seed potatoes (4°C), ware potatoes (8°C), and industrial processing (12°C). In a successive experiment, the viability of the surviving nematodes originating from potato tubers stored for 180 and 240 days at all temperatures was studied. Viability in this paper is defined as the ability to infect and reproduce on a potential host, in this case the susceptible cultivar Desiree. In addition, the population densities in the newly produced tubers were determined.

Materials and methods

Experiment 1: Source of infected tubers

Two weeks before planting, tubers of the susceptible cv. Desiree, stored at 4°C, were transferred to a shaded spot in the glasshouse at 18-20°C to initiate sprouting. When sprouts reached nearly 1 cm length, the tubers were exposed to full light to harden the sprouts.

One hundred pots, height 27 cm, diameter 25 cm, were filled with 10 kg dry soil mixture each (Teklu *et al.*, 2014). Pots were inoculated with a density of 12 J₂(g dry soil)⁻¹ with the *Meloidogyne chitwoodi* Mc31 ('Smakt') population, reared on the susceptible tomato cv. Moneymaker. Twenty injections per pot were made using a needle to inoculate the soil. With each injection, a 3 ml suspension of J₂ was gently released while withdrawing the needle from the soil. After inoculation, the holes were closed with soil and potato tubers were planted at the centre of the pot. The soil was covered with perforated black polythene sheet to avoid excess evaporation of the pots during the experiment and splashing during watering. The growth period of the potato was 21 weeks.

Processing tubers and Storage conditions

Approximately 30 kg of infected tubers were harvested. Tubers were collected from the soil, gently rinsed with water to remove any adhering soil, and dried for 48 h at room temperature. Tubers of all pots were combined and sorted into lots; based on tuber size and quality damage according to the classification criteria of Visser & Korthals (2004). Sorted tubers were divided into 13 lots composed of equal amounts (65-68) of tubers from each size and class to provide for 4 storage times (60, 120, 180, and 240 days) at three temperatures (4°C, 8°C and 12°C) and a control processed 48 h after harvest. Humidity of the storage facilities was maintained at 95 - 98% and adequate ventilation was provided. After each temperature-time treatment, tubers were kept for two weeks at 18-20°C to harden the skin before peeling.

Nematode extraction from the tubers

A layer of 5 mm (Viaene *et al.*, 2007) was peeled from all the tubers of each treatment. The peel was then cut into 1 cm² pieces, mixed and split into replications consisting of 30 g for the hatching test. The first lot, the control, consisted of 29 replications. The other 12 time and temperature combinations consisted of 11-12 replications. Each replication was placed on 20 cm diameter, 425 µm sieves. The sieves were then put on 25 cm diameter extraction dishes and kept in a spray-mist chamber for 7 weeks, while hatched J₂ were collected and counted every seven days. The chosen sieve aperture for tuber peel was larger than that for roots as used by Teklu *et al.* (2016b) to avoid clogging of the sieves by starch. Earlier studies (Teklu *et al.*, 2013) revealed a maximum number of hatched J₂ of *M. chitwoodi* at a peel weight of 0.045 to 0.113 g cm⁻² of sieve. A sieve of 20 cm diameter provides an area of 314.2 cm² and enables the processing of 30 g of peel. Temperature in the mist-chamber was kept

at 20°C and every 30 min a fine mist was applied to the peel for 15 min (Seinhorst, 1988). To adjust for possible differences in moisture content, each time a hatching test was started, left over peel from each time and temperature combination, ca. 50 g, was used to estimate dry weights of the peel by oven drying at 105°C overnight. Final population densities both per g of fresh or dry peel were estimated.

Experiment 2: Viability of J2s from tubers after 180 and 240 days of storage

Nematodes, surviving 180 and 240 days of storage, when the strongest effect of storage time is to be expected, were checked for their viability by testing their population dynamics on cv. Desiree immediately after they came out of storage. There were five replications (pots) per temperature time combination. Five kg of soil mixture (Teklu *et al.*, 2016b) was mixed thoroughly with 50 g of the tuber peel cuttings in a plastic bag and then filled into a 5-kg pot. Then, 15 mm x 30 mm (diameter x height) cylindrical, 12-g tuber cuttings of cv. Desiree were planted at 6 cm depth. The P_{peel} of the pots was estimated from an extra 50 g of tuber peel for each temperature time combination using the same method as described above and recalculated to $J_2(\text{g dry soil})^{-1}$.

Sixteen weeks after planting, shoots were cut, tubers were harvested and the roots were collected. Nematodes were extracted from both the roots and the soil to estimate the final population density (P_f) as describes by Teklu *et al.* (2016b). Tubers from the pot experiment, inoculated with the 180 days storage inoculum, were harvested earlier than those inoculated with 240 days storage inoculum and were stored at 4°C until the hatching test for both storage temperatures could be conducted. The extra storage times for tubers with $t_s = 180$ inoculum was 90 days. Tuber peel was collected and processed as described for Experiment 1.

Data analysis and model fitting

Data were analysed using scripts written in the Tinn-R editor version 4.00.03.5 and run in R version 3.3.2. Models were fitted to the data to estimate the parameters using ordinary Least Square Methods. The standard errors of the parameters were obtained from the inverse of the Hessian matrix (Venables & Smith, 2016).

Relative fresh tuber weight (relFTW) and storage time (t_s)

The relFTW was estimated by the ratio of fresh tuber weights after storage and at harvest. An exponential model, Equation (1), was fitted to the data to estimate the relative minimum FTW and the rate of weight decline.

$$\text{relFTW} = m_{\text{FTW}} + (1 - m_{\text{FTW}}) \times k^{t_s} \quad (1)$$

Where:

t_s	storage time (day)
m_{FTW}	minimum relFTW
k	the rate of decline

Hatching process of nematodes from tuber peel

When estimating the nematode numbers from the tuber peel, zero counts were encountered. These zeros were considered small numbers and were replaced by half of the minimum (hMin) of the non-zero values within a given set of replicates. When all replicates were zeros, the average was considered a true zero. In the regression analysis, these zeros were replaced by $1 \times 10^{-7} \text{ J}_2(\text{g dry soil})^{-1}$, a number that neither affected the parameter estimates nor their standard errors until the seventh decimal (Teklu *et al.*, 2017). After log transformation of the number of hatched J_2 , the means per set of replicates were estimated and back transformed. A logistic model, Equation (2), was fitted to the data and the three parameters; C , B and A were estimated.

$$F(t) = \frac{C}{1 + \exp(-B(t - A))} \quad (2)$$

Where:

- t Hatching time (days)
- C Maximum cumulative hatched $\text{J}_2(\text{g FPW})^{-1}$
- B Relative maximum hatching rate
- A Time when $F(t) = 0.5 \times C$ (days)

$P_{f_{\text{tubers}}}$ was converted from $\text{J}_2(\text{g FPW})^{-1}$ to $\text{J}_2(\text{g dry soil})^{-1}$ using total FPW and the total dry weight of soil (TSW).

The relations between the hatching parameters and storage time (t_s)

For the three storage temperatures, linear models, Equations (3) and (4), were fitted to the data, starting from $t_s = 60$ days. Before regression analysis, the estimates for parameter C , were log transformed. Then

$$\log C = \alpha \cdot t_s + \beta \quad (3)$$

It follows that

$$C = \exp(\alpha \cdot t_s + \beta) = \exp(\alpha \cdot t_s) \times \exp(\beta) \quad (4)$$

$$A \text{ or } B = \alpha \cdot t_s + \beta \quad (5)$$

Where:

- α coefficient of direction
- β_{60} intercept at $t_s = 60$ days
- t_s storage time (days)

An exception was made for the parameter A at 12°C . In this case, an exponential model, Equation 5, including a minimum, γ , for A , was more appropriate.

$$\log A = \alpha \cdot t_s + \beta + \gamma \quad (6)$$

The models only apply within the given ranges of the storage times, t_s . The parameter estimates immediately after harvest are independent of storage temperature and therefore excluded from data analysis.

Viability of nematodes after 180 and 240 days of storage

To assess the number of J2 at harvest of the viability experiment, numbers of J2 in the roots, soil and tubers were estimated. Equation (2) was fitted to the data of hatched nematodes from tubers and the three parameters C , B and A were estimated. As there were no significant differences between the use of fresh tuber peel or dry tuber peel, we prefer in the viability test $P_{f_{tub,gs}}$ (P_f from the tuber peel) expressed per g of dry soil. Estimates of $P_{f_{tub,gs}}$ and $P_{f_{r+s}}$ ($P_{f_{root}} + P_{f_{soil}}$) were plotted against $P_{i_{peel,gs}}$ and, if possible, Equation (7), (Seinhorst 1966, 1970) was fitted to the data. Full descriptions of the variables, parameters and their dimensions are presented Table 5.1.

$$P_f = M \times P_i / (P_i + M / a) \quad (7)$$

Where:

- P_i the population density at the time of planting J2(g dry soil)⁻¹
 P_f the population density at the time of harvest J2(g dry soil)⁻¹
 a the maximum multiplication rate
 M the maximum population density J2(g dry soil)⁻¹

Table 5.1. Description of the list of variables and parameters used.

Term	Description	Dimension
relFTW	Relative fresh tuber weight	--
DPW	Dry peel weight	g
FPW	Fresh peel weight	g
DM	Fraction of dry matter	--
TSW	Total dry soil weight in pot	g
m_{FTW}	Minimum relative tuber weight	--
K	The rate of decline	--
t_s	Storage time	day
T	Storage temperature	°C
hMin	Half of the minimum of non-zero values	J2(g FPW) ⁻¹
F(t)	Logistic model	J2(g FPW) ⁻¹
C	Maximum of F(t)	J2(g FPW) ⁻¹
B	Relative maximum rate of increase in F(t)	--
A	Time when F(t) = 0.5 × C	day
t	Hatching time	day
α	Coefficient of direction	--
γ	Minimum of A at T = 12 °C	day
β_{60}	Intercept at $t_s = 60$ days	--
P_i	Initial population density at planting	J2(g dry soil) ⁻¹
$P_{f_{root}}$	Final population density from roots	J2(g dry soil) ⁻¹
$P_{f_{soil}}$	Final population density from soil	J2(g dry soil) ⁻¹
$P_{f_{r+s}}$	Final population density at harvest	J2(g dry soil) ⁻¹
$P_{i_{peel,gs}}$	Initial population density from the peel	J2(g dry soil) ⁻¹
$P_{f_{tub,gs}}$	Final population density from tuber	J2(g FPW) ⁻¹
$P_{f_{tub,gs}}$	Final population density from tuber	J2(g dry soil) ⁻¹
a	Maximum multiplication rate	--
M	Maximum population density	J2(g dry soil) ⁻¹

Results

Weight loss of tubers during storage: Experiment 1

Relative tuber weights decreased during storage (Figure 5.1). The relation between relFTW and storage time, t_s , was well described by Equation 1 ($R^2 \geq 0.97$). The minimum relFTW (m_{FTW}) and the rate of decline, k , were the same for all storage temperatures: on average 0.58 and 0.99 respectively with coefficients of variation of 9 and 2%, respectively. Due to the weight decline, the dry matter content, DM, of the tubers increased from 0.21 at harvest time to 0.30 after 240 days of storage (Table 5.2). There was no difference between storage temperatures.

Early sprouting of tubers was noticed after 26 days at 12°C, and 60 days at 8°C. On tubers at 4°C, a few sprouts were noticed after 180 days of storage.

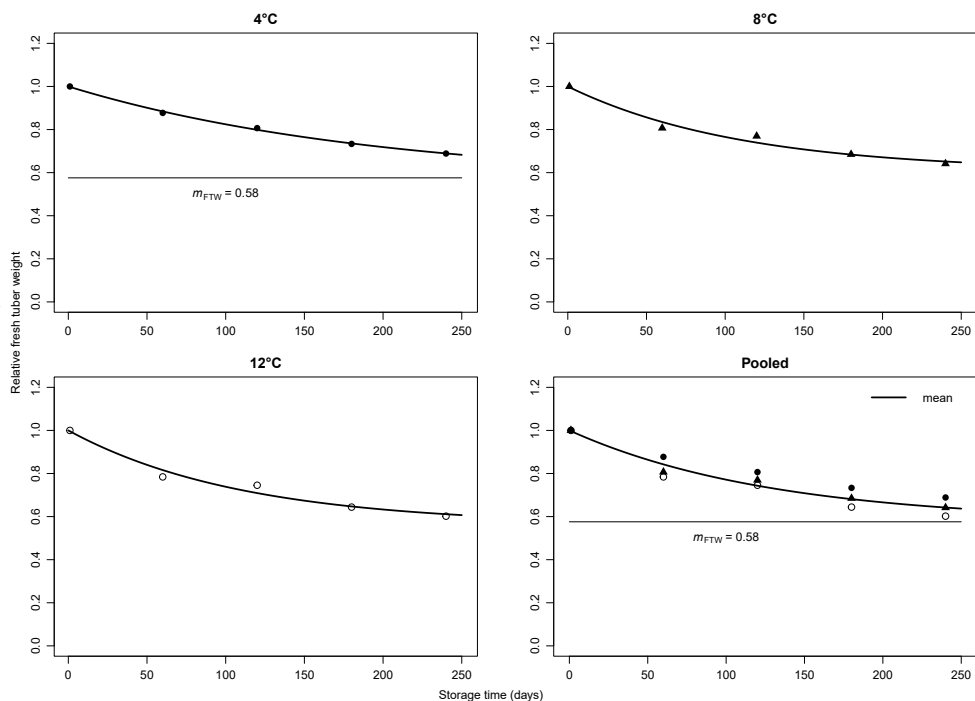


Figure 5.1 Experiment 1. The relation between relFTW and storage time t_s , at 4, 8 and 12°C. Fitted model: Equation (1) $\text{relFTW} = m_{FTW} + (1 - m_{FTW}) \times k^{t_s}$. Solid horizontal line (m_{FTW}) represents the minimum relative tuber weight.

Table 5.2. Experiment 1: Parameter estimates of the logistic model, Equation (2) $F(t) = \frac{C}{1 + \exp(-B(t - A))}$, fitted to the cumulative hatched J2 of *Meloidogyne chitwoodi* from the tuber peel for each storage time and temperature combination. Where: DPW= g Dry Peel Weight; FPW= g Fresh Peel Weight; DM = dry matter content as fraction; C= maximum cumulative hatched J2(g FPW)⁻¹; B = relative maximum hatching rate; A = time (days) when 0.5 × C is reached; SE = standard error of the parameters; R²= coefficient of determination and df = degrees of freedom.

Storage days	Storage temp (°C)	Rep	DPW (g)	FPW (g)	DM	C	B	A	SE _C	SE _B	SE _A	R ²	df
0	--	29	6.38	30	0.21	301.4	0.24	16.17	7.31	0.03	0.65	0.99	5
60	4	11	7.50	30	0.25	269.3	0.17	21.55	8.57	0.02	0.86	0.99	5
60	8	11	8.40	30	0.28	244.3	0.15	21.86	6.85	0.01	0.78	1.00	5
60	12	11	7.20	30	0.24	899.9	0.20	10.93	45.25	0.05	1.59	0.96	5
120	4	12	7.44	30	0.25	90.2	0.18	23.15	1.58	0.01	0.45	1.00	5
120	8	12	7.77	30	0.26	246.0	0.16	16.71	7.72	0.02	0.94	0.99	5
120	12	12	7.27	30	0.24	932.1	0.35	7.64	42.15	0.17	1.22	0.96	5
180	4	12	8.36	30	0.28	51.1	0.22	19.58	0.45	0.01	0.23	1.00	5
180	8	12	8.28	30	0.28	193.9	0.17	15.76	6.28	0.02	0.98	0.99	5
180	12	12	7.58	30	0.25	368.6	0.58	6.86	11.75	0.39	0.76	0.97	5
240	4	12	9.05	30	0.30	28.9	0.13	26.55	1.29	0.01	1.18	0.99	5
240	8	12	8.70	30	0.29	79.7	0.25	9.18	4.50	0.10	1.72	0.95	5
240	12	12	8.60	30	0.29	778.9	0.78	6.95	10.81	0.52	0.69	0.99	5

Nematode hatch from tuber peel: Experiment 1

Equation (2) fitted well to the data of the cumulative hatch (Figure 5.2). The parameter estimates and their standard errors are summarized in Table 5.2. The relations between the hatching parameters C, B and A and storage time, t_s , are visualized in Figure 5.3; their parameters are summarized in Table 5.3.

Table 5.3. Experiment 1. Parameters estimates of the relations between the hatching parameters of *Meloidogyne chitwoodi* C, B and A and storage time t_s . According to the log linear and linear Equations 4 and 5. Marked with an * is the relation between logA and storage time, t_s , at 12°C, where A reaches its minimum, $\gamma = 6.5$, according to Equation 6. β_{60} = intercept at $t_s = 60$ days, α = coefficient of direction, SE = standard error, CV = coefficient of variation. Parameter estimates of C, B and A at harvest, $t_s = 0$, were 301.41, 0.24 and 16.17, respectively.

Parameter	T	β_{60}	SE _{β_{60}}	CV _{β_{60}}	α	SE _{α}	CV _{α}
C	4	230.11	39.42	0.17	-0.0121	0.0015	0.13
	8	299.48	82.55	0.28	-0.0060	0.0024	0.40
	12	700.31	154.10	0.22	0.0000	NA	NA
B	4	0.19	0.04	0.19	-0.0002	0.0003	2.18
	8	0.14	0.02	0.16	0.0005	0.0002	0.39
	12	0.18	0.02	0.12	0.0033	0.0002	0.06
A	4	20.99	2.62	0.12	0.0190	0.0233	1.23
	8	21.73	1.37	0.06	-0.0649	0.0122	0.19
	12*	9.57	4.98	0.52	-0.0133	0.0044	0.33

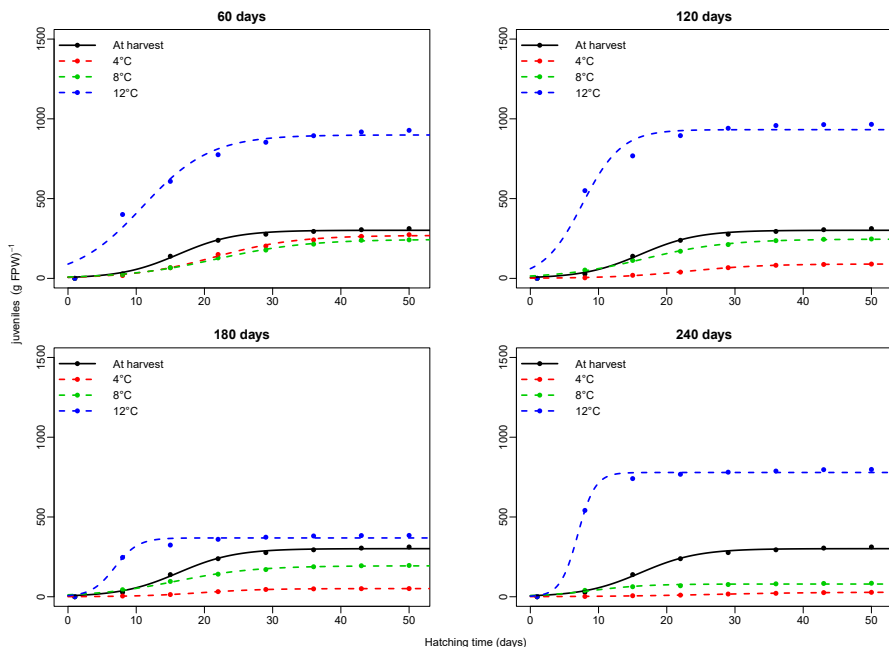


Figure 5.2 Experiment 1. The cumulative hatch of J2 of *Meloidogyne chitwoodi* at 4, 8 and 12°C after 60, 120, 180 and 240 days of storage. The solid black line is cumulative hatch immediately at harvest (or $t_s = 0$). Fitted model: Equation (2) $F(t) = \frac{C}{1 + \exp(-B(t - A))}$.

Parameter C: The maximum cumulative number of J2(g FPW)⁻¹

The logarithm of C changed linearly with storage time, t_s , at all storage temperatures (Equation 4). At a storage temperature of 4°C and $t_s > 60$ days C decreased at a rate of $\exp(0.012 \times t_s)$ or 1.012^{t_s} until 26.05 J2(g FPW)⁻¹ (9.1% of the estimate at harvest) at $t_s = 240$ days. At a storage temperature of 8°C, the decline rate of C was smaller than at 4°C, $\exp(0.006 \times t_s)$ or 1.006^{t_s} , and at $t_s = 240$ days C was estimated to be 79.7 J2(g FPW)⁻¹ or 35% of the numbers at harvest. In contrast to the lower storage temperatures, C at 12°C increased until 250% of the numbers at harvest during $0 < t_s < 60$ days; then remained more or less stable, on average 700.3 J2(g FPW)⁻¹ (Figure 5.3).

The coefficients of direction, α , increased at a rate of 0.0015 with storage temperature, T , until α equalled zero at $T = 12^\circ\text{C}$. Estimates of C from stored tubers at 4 and 8°C were the same at $t_s = 0$ and $t_s = 60$ days and averaged 262.5 J2(g FPW)⁻¹.

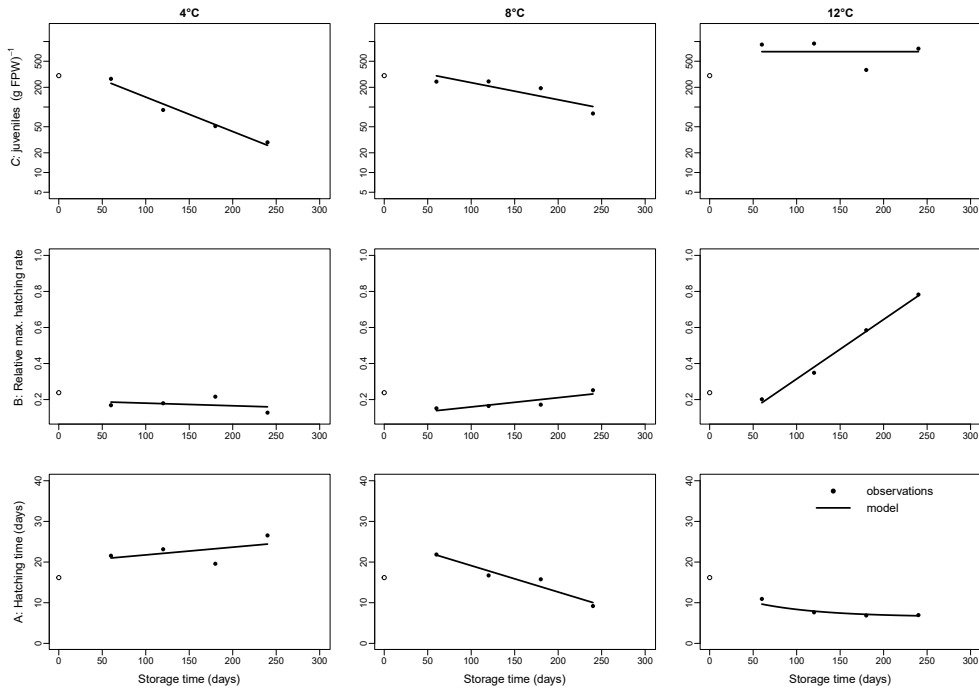


Figure 5.3 Experiment 1. The relation between the hatching parameters $\log C$, B and A and storage time, t_s , at 4, 8 and 12°C. The linear models, Equations (3) $\log C = \alpha \cdot t_s + \beta$ and (5) A or $B = \alpha \cdot t_s + \beta$, were fitted to the data. At 12°C the parameter A reached its minimum. Therefore, Equation (6) $\log A = \alpha \cdot t_s + \beta + \gamma$ was fitted. The open circle (o) represent the observation at harvest.

Parameter B: the relative maximum hatching rate

At all storage temperatures, B changed linearly (Equation 5) with t_s . At 4°C, B decreased slightly with $\alpha = -0.0002$ but at 8 and 12°C, conditions were favourable enough for B to increase with storage time at rates of 0.0005 and 0.0033 respectively. As for C , the coefficients of direction, α , increased linearly, at a rate of 0.0004 with storage temperature, T . At all storage temperatures, B at harvest ($t_s = 0$) was slightly larger than at $t_s = 60$ days.

Parameter A: Time when $F(t) = 0.5 \times C$ (days)

The time required for J_2 from tubers, stored at 4 and 8°C, to reach $0.5 \times C$, changed by $0.019 \times t_s$ and $-0.065 \times t_s$, respectively at $t_s > 60$ days (Equation 5, Figure 5.3). At 12°C, $\log A$ decreased at a rate of $\exp(-0.0133 \times t_s)$ or 0.986^{t_s} at $t_s > 60$ days until 6.5 days (43%) at $t_s = 240$ days (Equation 6). While A increased by 1.4 times at $0 < t_s \leq 60$ at storage temperatures of 4°C and 8°C, at 12°C it decreased during the same storage interval to 70% of its value at harvest ($t_s = 0$).

Infectivity and viability of J2s after storage: Experiment 2

Nematodes surviving in tubers stored for 180 and 240 days, at all storage temperatures, were able to reproduce on roots and tubers of cv. Desiree. Initial and final population densities of *M. chitwoodi*, expressed as $P_{i_{\text{peel.gs}}}$ and $P_{f_{r+s}}$ respectively, are presented in Table 5.4.

Table 5.4. Experiment 2. Initial population densities, $P_{i_{\text{peel.gs}}}$, and final population densities, $P_{f_{r+s}}$ and $P_{f_{\text{tub.gs}}}$, of *Meloidogyne chitwoodi* at harvest of the viability pot test with cv. Desiree. $P_{i_{\text{peel.gs}}}$ is obtained from tubers after 180 and 240 days of storage at 4, 8 and 12°C.

Storage time, t_s in days	Storage temperature, T in °C	$P_{i_{\text{peel.gs}}}$	$P_{f_{r+s}}$	$P_{f_{\text{tub.gs}}}$
180	4	0.51	39.18	0.34
180	8	1.96	34.09	1.29
180	12	3.85	68.10	0.82
240	4	0.28	11.01	5.61
240	8	0.85	24.47	1.75
240	12	7.98	59.30	3.19

Nematodes from root and soil: $P_{f_{r+s}}$

Equation (7) was fitted to the $P_{i_{\text{peel.gs}}} \sim P_{f_{r+s}}$ data (Figure 5.4) and the maximum multiplication rate (a) and maximum population density (M) were estimated to be 63.6 and 70.8 J2(g dry soil)⁻¹ respectively; the M/a ratio equalling to 1.1. The 2.5% and 97.5% quantiles of the two parameters were almost the same: 26 and 153 for a and 32 and 156 for M .

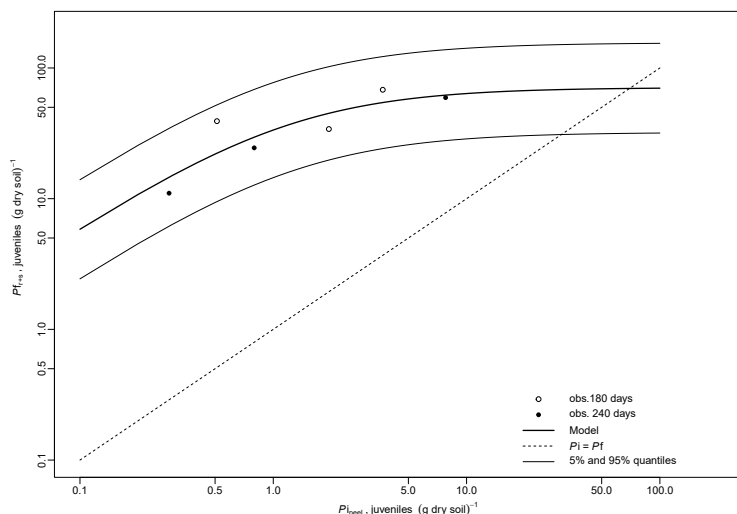


Figure 5.4 Experiment 2. Relation between $P_{i_{\text{peel.gs}}}$ and $P_{f_{r+s}}$ of *Meloidogyne chitwoodi* on cv. Desiree in the viability test. The diagonal dotted line represents the equilibrium densities where $P_i = P_f$. The thick and thinner solid lines are according to Equation (7) $P_f = M \times P_i / (P_i + M / a)$: representing the 2.5, 50, and 97.5% quantiles.

Nematodes from tuber peel: $P_{f_{tub, gp}}$

The hatching process of the nematodes in the tuber peel of Experiment 2 of the viability test was modelled using Equation 2 to estimate $P_{f_{tub, gp}}$, and visualized in Figure 5.5 when expressed as $P_{f_{tub, gs}}$. No regression was found between P_i and any of the hatching parameters (Figure 5.6). Parameter B was the same for J2 originating from storage periods $t_s = 180$ days and $t_s = 240$ days ($P = 0.23$). This was not true for the maximum cumulative hatch C , the estimator of $P_{f_{tub, gs}}$, and A ($P < 0.05$). The $P_{f_{tub, gp}}$ of nematodes originating from tubers stored for 180 days was 23% compared to that of nematodes from tubers stored for 240 days. At the same time, the nematodes from 180 days stored tubers needed an extra 6 days $2 \times A$, ($= 29\%$), to reach their maximum cumulative hatch, C (Figure 5.6).

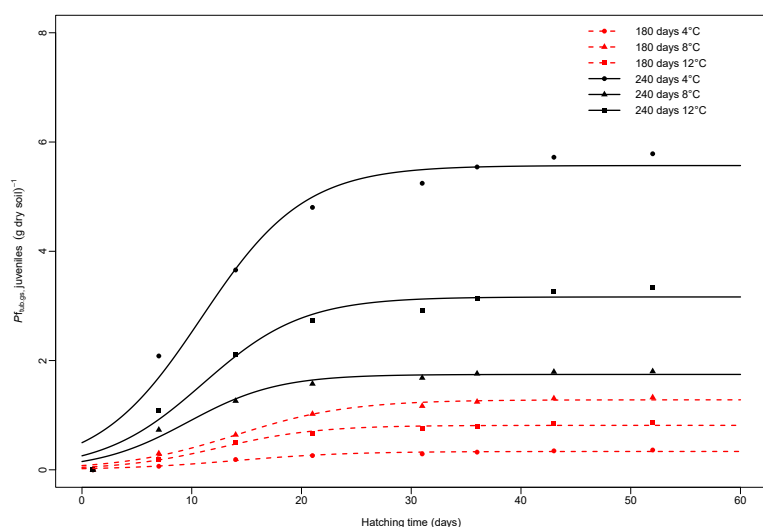


Figure 5.5 Experiment 2. The cumulative hatch of J2 of *Meloidogyne chitwoodi* from peel at harvest of the viability test. Initial population densities originated from tubers stored for 180 and 240 days at 4, 8 and 12°C in Experiment 1. Fitted model: Equation (2) $F(t) = \frac{C}{1 + \exp(-B(t-A))}$.

All multiplication rates on the tubers of Experiment 2, inoculated with J2 from $t_s = 180$ days were ≤ 0.66 , while two out of three multiplication rates of J2 from 240 days stored tubers were > 2 (Figure 5.6). The $P_{f_{tub, gp}}$, varied between 49 and 122 J2(g FPW) $^{-1}$ or 0.34 till 1.28 J2(g dry soil) $^{-1}$ for J2 on 180 days stored tubers. For J2 from 240 days stored tubers, these numbers were higher, varying between 262 and 453 J2(g FPW) $^{-1}$ or 1.7 and 5.6 J2(g dry soil) $^{-1}$ (Table 5.4).

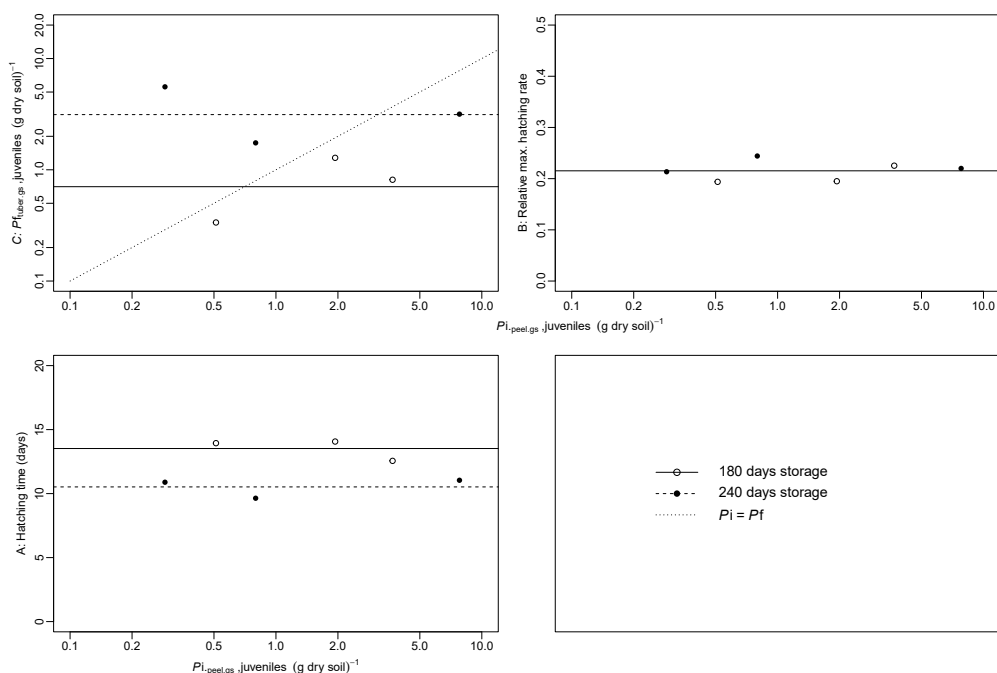


Figure 5.6. Experiment 2. Relation between initial population densities, $P_{i, \text{peel,gs}}$, and parameters C ($P_{\text{tub,gs}}$), B and A of the logistic hatching curve of *Meloidogyne chitwoodi* on cv. Desiree in the viability test. Parameter C graph: the diagonal dotted line represents the equilibrium densities were $P_i = P_f$; the horizontal solid and broken lines indicate the maximum population densities, M , for P_i from tubers, after storage times, t_s , of 180 and 240 days respectively. Parameter B and A graphs: the horizontal solid and broken lines indicate the nematodes originating from $t_s = 180$ and 240 days, respectively.

Discussion

Weight loss and sprouting: Experiment 1

Weight loss during storage was positively correlated with increasing storage time but not with temperature. This is in accordance with Schippers (1971), who reports that time rather than temperature is the major factor in weight loss of tubers in storage. According to Burton (1966) and Schippers (1971), weight loss is caused by transpiration and sprouting. Mehta & Kaul (1988) report a 9-10% weight loss after 60 days of storage at 2-4°C and a RH of 90-95% for three different, not nematode infected, cultivars. That is only slightly lower than the 12% weight loss after 60 days at 4°C in this experiment. But lower weight losses, 1-3% after 60 days of storage have also been reported, e.g. Butchbaker *et al.* (1973) and Singh *et al.* (1975).

Earlier sprouting at higher temperatures is the main reason to store tubers at low temperatures, a fact that was confirmed in this experiment. Nourian *et al.* (2003) reported sprouting of tubers of cv. Chieftain after 62 days at 12°C, 112 days at 8°C and no sprouts until

135 days at 4°C at RH of 95%. Others, e.g. Burton (1973), also noted the early sprouting noticed at 12°C. The latter observed cv. Chieftain sprouting after 62 days at 12°C; after 112 days at 8°C and after 135 days at 4°C at a RH of 95%. The comparatively earlier sprouting of cv. Desiree might be due to cultivar differences (Lisinska & Leszczynski, 1989).

There was some doubt whether or not tuber shrinkage, especially after $t_s = 180$ days, would cause the collected 5 mm peel to cover a larger part of the tuber than immediately after harvest and, as a result, increase the number of $J_2(g\text{ FTW})^{-1}$. However, this was not confirmed by the data.

The Hatching process after storage: Experiment 1

From the hatching curves it can be concluded that the hatching process of the nematodes from the peel of the stored tubers speeded up with increasing storage time, following mostly a log-linear pattern, causing the relative maximum hatching rate (B) to increase and the time ($2 \times A$) to complete the hatching process, to decline. Meanwhile, the decline of the total number of hatched nematodes, (C), during storage time lessened with storage temperature, until, at 12°C, it stabilized, after an increase during the early storage period.

Not only $\log C$, B , and A are linearly related to storage time, t_s - except at 12°C, where A finds its minimum - also their rate of change, α , is linearly related to storage temperature, T . This means that within the given ranges of storage time and storage temperature, we can predict C , the number of $J_2(g\text{ FPW})^{-1}$, the duration, $2 \times A$, and the relative maximum hatching rate, B , of the hatching procedure under the given conditions. Beyond the boundaries of the storage temperatures and times, the parameters' change will be bound to limitations: C cannot go on decreasing, B cannot increase endlessly and A already found its limit at 6.5 days at 12°C.

The decrease of C , the number of $J_2(g\text{ FPW})^{-1}$, during storage time at 4°C coincides with an increase in tuber sugar content (Matsuura-Endo *et al.*, 2014). This happens in all potato cultivars, although sugar levels vary between cultivars and depend on growing conditions (Mc Kenzie *et al.*, 2005). Although there is no direct proof or a conclusive explanation of the physiological process, declining nematode numbers during storage at low temperatures are often attributed to the high sugar levels, e.g. by Olthof & Yu (1999). In the case of *Meloidogyne*, increasing sugar levels, causing increasing distortion of hydration during the hatching process after storage (Wright, 1998; Perry *et al.*, 2013) could result in nematode decline. An argument against this theory is that under the mist chamber conditions the tuber peel is constantly washed by the spray the sugar levels are diluted. Whether or not this is sufficient for an undisturbed hatching process remains to be investigated.

Another cause for the nematode decline during storage could be the existence of a normal distribution of the sensitivity of nematodes to unfavourable conditions, e.g. pesticides. Sensitive nematodes die immediately after exposure while nematodes that are more tolerant endure these conditions much longer. This results in a logistic relation between pesticide

concentration and nematode survival (Schomaker & Been, 1999). Similarly, there could be a logistic relation between low storage temperature and nematode survival. As we do not have observations over the whole range of storage time, we could only observe the linear part of the logistic model. At 8°C storage temperature either theory can occur, only at a lower level as is indicated by the estimates of C , A and B . The temperature is favourable enough to let part of the immature eggs ripen until the J2 stage during storage, resulting in a shortening hatching process with lesser time to reach half of the maximum hatch (A) and a larger relative maximum hatching rate (B).

At the storage temperature of 12°C the $P_{f_{tubers}}$ increases 2.5 times, while the hatching process speeds up, resulting in four times increase in B and a simultaneous decrease in A until its limit, 6.5 days, is reached, implying that at $120 < t_s < 240$ the hatching process is completed in about 13 days. In our graph, B at $T = 12^\circ\text{C}$ keeps increasing steeply, but logically at some point in storage time – beyond our data set – this increase must be limited. Females laying eggs during the early stages of storage probably cause the increase in $P_{f_{tubers}}$ at $t_s > 60$ days. During the whole storage period, ripening of immature eggs progresses, completing their life cycle until J2. As practically all nematodes will be at the same stage at the end of the storage period, hatch is almost immediate at hatching days > 120 .

Practical implications

The smaller or higher storage temperatures, 4 and 12°C, cause $P_{f_{tuber}}$ to decrease or increase, respectively. At only one storage temperature, 8°C, $P_{f_{tuber}}$ (C) remains approximately at the same level as immediately after harvest for at least until 120 storage days. This information is especially important for the conductance of greenhouse and field tests, as storage of organic material with nematodes is unavoidable because of limitations to processing capacity in laboratories. At 8°C, the time needed to complete the hatching process ($2 \times A$) decreases with t_s and amounts to 18 days when $t_s = 240$ days.

For agricultural purposes, the decline in nematode numbers at 4°C is important as the transfer of *M. chitwoodi* by seed potatoes is reduced until less than 10% after 240 days of storage, which covers the majority of storage times in practice. Seed potatoes, which are harvested earlier than ware potatoes and potatoes for industrial processing, might be stored a little longer. However, for the susceptible cv. Desiree, this reduction is not sufficient to prevent severe quality damage, as up to now, its tolerance limit appeared to be unmeasurable, but smaller than $0.0625 \text{ J2(g dry soil)}^{-1}$ (Teklu *et al.*, 2016a).

It is also evident that although densities might decrease in storage, spread of this plant pathogen is not stopped in the case of a susceptible potato cultivar. The prospects of cultivars that are both resistant and tolerant to *M. chitwoodi*, e.g. the clone AR04-4096 (Teklu *et al.*, 2016b) with relative susceptibilities (RS), both RS_{r+s} and $RS_{tuber} < 1\%$ and $T > 10 \text{ J2(g dry soil)}^{-1}$, are more hopeful and should be investigated, especially under the population densities encountered under field conditions.

The official EPPO protocol, testing 200 randomly sampled tubers after harvest and incubating them at temperatures around 18°C until a minimum temperature sum of 2150 day-degrees is reached, is inadequate. It also does not discriminate between symptoms and live or dead nematodes; symptoms might increase during storage (Finley, 1981), but the number of live nematodes can decrease or increase during storage before sampling depending on storage temperature. When considering the high degree of resistance in the newly developed potato genotypes (Teklu *et al.*, 2016b) and the low numbers found in the tubers of these resistant genotypes this might become an issue of consideration in the future.

Infectivity of J2s after storage: Experiment 2

The surviving nematodes, obtained after 180 and 240 days at all storage temperatures, were still able to infect roots and tubers of a next potato crop. The surviving J2 multiplied on cv. Desiree and caused severe quality damage to the tubers. The population dynamic parameters in the relation $P_{\text{peel,gs}} \sim P_{\text{fr+s}}$ were similar to those reported on cv. Desiree by Teklu *et al.* (2016b) when fresh J2 were used, although the 95% confidence limits in this experiment, partly caused by the limited number of data points, was much larger.

Surprising was both the increase and decrease of A and C , respectively, of the P_{ftubers} , inoculated with nematodes originating from 180 days stored tubers compared to those stored for 240 days. We would expect a larger P_{ftubers} originating from the inoculum of the tubers stored for 180 days than from inoculum from tubers stored for 240 days, at least from those inoculated at 4 and 8°C. The difference is probably best explained by the longer intermediate storage time of 90 days at 4°C of the tubers stored from the pot test inoculated with J2 from $t_s = 180$ days, before the peel was extracted in the mist chamber. According to the data from Experiment 1, at a storage temperature of 4°C we can expect C to decrease and the time $2 \times A$, needed the nematodes to reach their maximum cumulative hatch, to increase. In our storage Experiment 1, C decreased to 30% between 60 and 120 days of storage, while A increased by 40%. These figures are close to those in the viability test and confirm that the same reduction during storage occurred in Experiment 2 as in Experiment 1. Future experiments could be improved by increasing the storage temperature of nematodes in tubers to 8°C where C , the estimator of P_{ftubers} , remains constant until 180 storage days. The slight decrease in B is not relevant, as A determines the prolongation of the hatching test.

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Chapter 6

Relative susceptibilities of five fodder radish varieties (*Raphanus sativus* var. *Oleiformis*) to *Meloidogyne chitwoodi*

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The fodder radish varieties Anaconda, Contra, Defender, Doublet and Terranova, known to have some resistance to *Meloidogyne chitwoodi* were compared to the standard susceptible variety, Radical, to estimate their relative susceptibility (RS) for both population dynamics parameters of *Meloidogyne chitwoodi* and evaluate P_i dependency. This approach must eventually lead to new screening methods for resistance tests. Plants were grown under controlled glasshouse conditions. Twelve densities of nematodes in five replications were used. Five plants per 7 l pot were grown for a period of 11 weeks, until their early flowering stage. A few seedlings of all the varieties at $P_i = 32$ and $64 \text{ J}_2(\text{g dry soil})^{-1}$ and all seedlings exposed to the highest density, $P_i = 128 \text{ J}_2(\text{g dry soil})^{-1}$ died within a week after germination. Replanted seedlings developed into normal plants. Total yield, expressed as total fresh weight, was not affected by *M. chitwoodi*. A lower percentage of plant roots with galls were observed on resistant varieties compared to Radical. For Radical a maximum multiplication rate (a) of 0.38 and a maximum population density (M) of $6.43 \text{ J}_2(\text{g dry soil})^{-1}$ were estimated. Radical proved to be a bad host for *M. chitwoodi* with all final population lower than the P_i . The parameter estimates of M for Anaconda, Contra, Defender, Doublet and Terranova were 0.011, 0.006, 0.027, 0.020 and 0.009 $\text{J}_2(\text{g dry soil})^{-1}$, respectively. With Radical being 100% susceptible, this resulted in RSM values of 0.17, 0.10, 0.42, 0.32 and 0.14% of these varieties, respectively, reducing high population levels of *M. chitwoodi* with more than 98%. There was no correlation between the rM_{galls} and the RSM values, indicating that scoring the number of galled roots will not provide a suitable measure for resistance.

Keywords: Host-status, maximum multiplication rate, maximum population density, resistance testing, root-knot nematode.

Introduction

Among the numerous species of plant-parasitic nematodes described, root-knot nematodes (*Meloidogyne* spp.) are important agricultural pests, attacking many mono and dicotyledonous plant species (Santo *et al.*, 1980; Kuttywayo & Been, 2006). More than 2000 species have already been identified as hosts for root-knot nematodes (Bird *et al.*, 2008) and more than 90 species of root-knot nematodes are described so far (Hunt & Handoo, 2009). Ten of these species are of importance from an agricultural point of view, and four species, *Meloidogyne hapla*, *M. arenaria*, *M. javanica* and *M. incognita*, are considered as major pests in many parts of the world (EPPO/OEPP, 2004). Root-knot nematodes cause considerable problems in intensive agricultural cropping (Caillaud *et al.*, 2008) and can cause substantial economic losses, mainly due to quality damage, to annual tuber crops (Boydston *et al.*, 2007; Bird *et al.*, 2008).

Meloidogyne chitwoodi was first described from Pacific Northwest of the USA (Santo *et al.*, 1980). However, the first detection occurred much earlier, as re-examination of old specimens and taxonomic illustrations showed that *M. chitwoodi* was present in The Netherlands in the 1930's (EPPO/OEPP, 1991, 2004; Karssen, 1995, 1996, 2002). *Meloidogyne chitwoodi* is, so far, found in North America (USA and Mexico), South America (Argentina), Africa (South Africa and Mozambique), Europe, Australia and New Zealand. It is listed as a quarantine organism within the European Union and is officially reported in Belgium, France, Germany, Sweden, Portugal, Turkey and The Netherlands (EPPO, 2013). Its status as a quarantine organism in Europe is mainly in order to prevent its further spread to non-infected areas (Smith *et al.*, 1997).

Due to its multiple host range, which in addition to arable crops includes numerous weeds and green manure crops, this plant-parasitic nematode is difficult to contain. Green manure crops, which are increasingly grown to restore organic matter, prevent nitrogen leaching and contain the spread of soil-borne diseases by dust and storms, may counteract the normal winter decline of *M. chitwoodi*. The resulting, comparatively high population densities in spring cause yield losses, mainly quality loss, often even at very small densities *e.g.*, three second-stage juveniles (J₂) (100 g dry soil)⁻¹ in carrots (Wesemael & Moens, 2008). As a result, *M. chitwoodi* is becoming more notable in the temperate climatic zones. Currently, *M. chitwoodi* causes quality loss in subterranean parts of several plants, including economically valuable crops such as potato (*Solanum tuberosum*), carrots (*Daucus carota*) and black salsify (*Scorzonera hispanica*) (Santo *et al.*, 1980; O'Bannon *et al.*, 1982; Brinkman *et al.*, 1996; Wesemael & Moens, 2008). In potato, *M. chitwoodi* causes prominent pimple-like blisters and discolouring of the flesh in tuber (Moens *et al.*, 2009). Some potato varieties are known to show only limited galling on the surface of the tubers but may show serious necrosis inside the tuber (Abad *et al.*, 2003). In areas like the Pacific Northwest of the USA, where this nematode was first described, economic losses of about \$40 million per annum are caused (Santo, 1994).

As nematicides cannot reduce nematode densities sufficiently (Molendijk, pers. comm.), the use of resistant varieties should play a prominent part in the management of this plant-parasitic nematode (Stirling, 2002; Melakeberhan *et al.*, 2006). One of the important green manure crops, also grown widely in The Netherlands, Germany and France, resistant to *M. chitwoodi* and beet cyst nematodes, is fodder radish (Al-Rehiyani & Hafez, 1998a; Koch *et al.*, 1999; Koch & Gray, 2002). Fodder radish is known for its fast germination and quick soil coverage. It also possesses a root system that can grow more than 2 meter deep (Walter, 1984). For that reason, apart from being a trap crop to plant-parasitic nematodes (Jackson *et al.*, 1993; Kristensen & Thorup-Kristensen, 2004), fodder radish has also been described as a catch crop or nitrogen scavenger.

Currently, screening for resistance to *Meloidogyne* is mainly done by rating gall formation and/or counting and comparing the egg masses (McSorley & Frederick, 1995; Van der Beek & Mugniéry, 2008; Wesemael & Moens, 2012). This method presents no quantitative information on the final population density (P_f) as the number of developed infective J2 is still unknown. It is very difficult to estimate the exact number of eggs in the gelatinous matrix, by either counting knots or staining of eggs masses. In addition, the number of J2 in the mineral fraction (soil) is disregarded, which can be quite large for crops with decreasing roots system well before the time of harvest, like potato. In addition, some of the screening procedures are too short for the nematode to finish its life cycle (Zoon *et al.*, 2003; Visser *et al.*, 2008). Overall, in these tests the estimation of the actual P_f , the maximum multiplication rate or the maximum population density is hampered and, consequently, the reliability of the estimated degree of resistance is questionable.

Therefore, an efficient testing method must be developed to obtain an accurate estimate of the degree of resistance. It must be an estimator derived from population dynamic parameters, which can be used to quantify the effect of rotation schemes in nematode management strategies. Such a measure, the 'relative susceptibility' (RS) has been successfully applied for potato and potato cyst nematode and lead to a tremendous reduction in agro-chemicals use and yield losses in The Netherlands. As the RS of potato cultivars with resistance to *Globodera pallida*, pathotype Pa3, is in most cases P_i independent (Phillips, 1984; Seinhorst & Oostrom, 1984; Seinhorst *et al.*, 1995), it seemed reasonable to screen potatoes at one P_i value. This implies that the RS_a (a = maximum multiplication rate) equals RSM (M = maximum population density). With population dynamic studies of *G. pallida*, pathotype Pa3 on susceptible cultivars and *G. rostochiensis*, pathotypes Ro1 and Ro2, Seinhorst (1984) showed that P_i independency of RS cannot be taken for granted.

To use RS values for fodder radish varieties, it is necessary to investigate the P_i independence of the resistance of these varieties to *M. chitwoodi*. Moreover, unlike potato clones, where each tuber has the same resistance, seeds of fodder radish are mixtures of resistant and susceptible genotypes, probably due to the out-crossing descent (Zoon *et al.*, 2003). It has to be researched how this influences the variation of the estimated RS value. This paper is therefore designed to explore both questions. Further, it should provide more insight into the possibility of developing a simpler and cheaper routine resistance tests for fodder radish.

Materials and methods

Experimental setup

To study the relation between the initial population density (P_i) and the final population density (P_f) of *M. chitwoodi* on fodder radish, six varieties were grown in a climate controlled glasshouse. Nematode densities at a geometric series, ranging from 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, to 128 J2(g dry soil)⁻¹ (Greco & Di Vito, 2009; Norshie *et al.*, 2011) were used. Each density was tested in 5 replications, except $P_i = 128$ which was in 3 replications. Five pots with the susceptible tomato cv. Moneymaker were used to check the pathogenicity of the inoculums at $P_i = 4$ J2(g dry soil)⁻¹. All plants were grown in 7 l plastic pots, in total 353 pots, that were randomly allocated to bench positions in the glasshouse were used. The plants received 16 h of daylight: 400 W, 58 500 lumen lamps at 18°C, while night temperatures were kept at 15°C. The humidity in the glasshouse was maintained at about 68%, concurrent with normal fodder radish growing conditions in The Netherlands.

Soil mixture

In order to make sure that *M. chitwoodi* was the only pathogen present in the experiment a soil mixture, consisting of pre-glacial silver sand, hydro-grains and clay powder in the proportion of (6:1.5:1) was used. Nitrogen, Potassium and Phosphorus (NPK) in the ratio of 14:13:14 and Steiner-nutrient solution of electrical conductivity (EC=2) for the supplementation of trace minerals such as of Ca, Mg, S, Zn, Cu and Na were added (Steiner, 1968). Batches of 1000 kg of soil mix were prepared composed of 700 kg silver sand, 180 kg hydro-grains, 120 kg clay powder, 1 kg coated-grains of NPK, 20 l of Steiner-nutrient solution and 38 l of water. Each heap was shoveled four times in order to ensure the homogeneity of the components. One kg sub-sample was taken from each heap and exposed for 24 h to a temperature of 105°C to determine the amount of moisture. Each pot was filled with 5.9 kg dry weight of the soil mixture and moisture content was later on adjusted to 10%.

Fodder radish varieties, pots and planting

Original stocks of seeds of fodder radish varieties were supplied by Joordens Zaden (Anaconda, Doublet, Radical and Terranova) and P.H. Petersen Saatzuch (Contra and Defender).

The pot size of (base diameter 16 cm x top diameter 20 cm x height 27 cm) was selected according to the root type of fodder radish. It usually has a long taproot and thus the pots were filled with the soil mixture to about 24 cm of the pot height. A filter paper was placed over the perforated base to avoid leaching of the soil mixture. The pots were filled with the soil mixture in 4 layers, with each layer being gently compressed, until the required final weight was attained. All pots were labelled, covered with white plastic to prevent evaporation and stored in the glasshouse until the date of inoculation of the infective J2.

According to breeder's information, the seeding rate (SR) of fodder radish is 20 kg ha^{-1} , which is 2 g m^{-2} . The thousand kernel weight (TKW), of seeds is *ca.* 13 g. Based on this, for a given surface area (SA) of 0.0314 m^2 , $(1000 \times \text{SR} \times \text{SA})/\text{TKW}$, 5 seeds are required to obtain the same plant density as in the field. Seeds were planted at a depth of 2 cm. Based on a germination rate of seeds ($> 80\%$); another 25% of the required numbers of seeds were sown on separate seedbeds to replace non-germinating seeds in the pots. The maximum age of these seedlings was 7 days.

Nematode inoculum and Inoculation

Meloidogyne chitwoodi population ("Smakt" Mc31), obtained from HZPC Research, was reared on the susceptible tomato cv. Moneymaker, grown in (base & top diameter 22 cm x height 27 cm) plastic pots filled with 5 kg silver sand. After the tomato plants had fully grown, the stems were removed and the fully galled roots were sieved over a 10 mm mesh, which withholds the roots but allows the silver sand to pass. The roots were then cut into 1 cm pieces and placed in the Seinhorst spray-mist chamber to extract the infective J2. The obtained nematode suspensions were stored at 4°C and continuously supplied with oxygen. Only the J2 collected during the first week were used. In total 56, 5-kg-pots were required to obtain the entire inoculum.

The suspensions for all individual densities were prepared from one stock solution and stored in separate bottles prior to inoculation. For inoculation, ten 30 cm long needles were placed equidistantly in each pot. Then, a 3 ml-pipette with the required amount of infective J2 was placed on the needles and its content was gently released while the needles were slowly pulled up. This ensures approximately random distribution of the J2 along the vertical profile of the soil (Been & Schomaker, 1986). To prevent desiccation of the J2, the needle holes were carefully covered with soil. The control for each variety was treated in the same way using tap water.

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Watering and fertilizing

Before planting, the amount of moisture in each pot was adjusted to about 10% of the total dry weight of the soil. When the seedlings emerged the amount of water was adjusted to 15%. Each week, pots were weighed on a computer-controlled scale and pots were watered up to 12-15% moisture content. At the same time, the pots were rotated, in order to avoid positional effects. In between the watering dates, when evaporation was high, five pots were selected at random and weighed and a fixed volume of water, consistent with the estimated weight loss, was given to each pot.

Measurements

Each week the evaporation was measured to estimate the water use of each pot. At harvest, the fresh and dry weights of shoots and roots were determined and the presence of galls was recorded for each plant. The final population density was estimated from the organic fraction (roots) and the mineral fraction (soil) per pot. To estimate population densities in the roots a spray-mist chamber (Seinhorst, 1988) was used. Roots were gently washed with tap water to remove any adhering soil, chopped into pieces of approximately 1 cm and transferred to 150 µm sieves with a diameter of 9 cm. The nematodes were collected weekly for 4 weeks and kept at 4°C until counting. For the mineral fraction, the soil in each pot was mixed carefully and a sub-sample of 500 g was taken and elutriated using Seinhorst elutriators (Seinhorst, 1962, 1988). The final population density was expressed as $J_2(\text{g dry soil})^{-1}$ and consisted of the sum of the nematodes in the mineral and the organic fraction. A nematode count of at least 200-300 was made throughout the experiment in order to minimize variations at different nematode densities.

Data analysis and Modeling

Population dynamics

Data analysis and fitting of the model was done using scripts written in Tinn-R Version 2.3.5.2 and run in R-version 3.0.1. The replicates of the final population density were log transformed, averaged and back transformed. Zeros were replaced by half of the minimum of a set of replicates. These values were not considered “true” zeros, but only samples from distribution functions that contain very small numbers. This is especially true at small P_i values. The population dynamics model for migratory nematodes, developed by Seinhorst (1970) and as described by Schomaker & Been (2013), was compared to the model for sedentary nematodes with one generation. The first model (Equation 1) fitted best to the data and was used to estimate the parameters maximum multiplication rate (a) and maximum population density (M) in $J_2(\text{g dry soil})^{-1}$, using the Least Squares Method.

$$P_f = M \times P_i / (P_i + M / a) \quad (1)$$

Standard errors and 95% confidence intervals of the parameters were estimated using the inverse of the Hessian matrix, while goodness of fit was evaluated by R^2 , adjusted for the degrees of freedom. Least Significant Differences (LSD) were calculated to evaluate differences between either parameters or variables. For (a) and (M) the differences and LSD of the log transformed estimates was taken. If in the next sections “difference” or “no difference” are mentioned, this indicates a statistical significant difference at $P = 0.05$. Resistance was expressed as Relative Susceptibility (RS): the ratios of identical population dynamic parameters of the resistant varieties and that of standard variety, Radical (Phillips, 1984; Seinhorst, 1984; Seinhorst *et al.*, 1995).

$$RS = a_{res}/a_{sus} \text{ or } M_{res}/M_{sus} \quad (2)$$

The deviations, D , of the P_i observations and the model, both log transformed, were analyzed separately. Plotting these deviations against the P_i made clear that the variation in the P_i at P_i values (≤ 2) were underestimated because of the many zero counts at these densities. Therefore, P_i values at (≤ 2) were removed from the analysis. For D -values at P_i (> 2), the mean and the standard deviation were calculated.

Results

Germination and sudden death

On average 23.6% of the seeds per variety, either did not germinate or seedlings died after germination within 7 days after sowing. This includes a few seedlings at $P_i = 32, 64$ and all seedlings exposed to the highest density of 128 J2(g dry soil)⁻¹. An overview of the germination data is provided in Table 6.1. The nematodes at densities from 0.125 to 16 J2(g dry soil)⁻¹ did not affect the emergence and development of the fodder radish seedlings, in contrast to plants at higher nematode densities. After 7 days, 7-day-old seedlings replaced all non-germinated seeds or dead seedlings. From that date, all fodder radish plants developed normally and flowered from 6 - 8 weeks on after planting. However, flowering time differed among the varieties. It was 42 for Anaconda and Terranova, 48 for Doublet, 54 for Defender and Radical and 64 days for Contra. After 11 weeks, all plants were harvested.

Table 6.1. Number of non-germinating seeds and seedlings suffering “sudden death” replaced per 25 plants per density per variety, except at $P_i = 128$ with three pots and 15 plants per variety. In total 290 plants were grown of each variety. On average 23.6% of the seeds were replaced by 7-day-old seedlings. J2= second-stage juveniles of *Meloidogyne chitwoodi*. P_i is measured in J2(g dry soil)⁻¹.

	Variety					
P_i (J2)	Anaconda	Contra	Defender	Doublet	Terranova	Radical
0	2	2	4	3	3	3
0.125	6	2	4	4	6	2
0.25	9	4	2	4	7	3
0.5	5	1	1	4	8	4
1	3	8	5	3	1	1
2	7	4	3	4	3	3
4	4	2	5	2	3	2
8	5	5	4	8	8	2
16	8	3	1	8	6	5
32	8	8	7	7	3	4
64	11	12	9	6	11	5
128	15*	15*	15*	15*	15*	15*
Total	83	66	60	68	74	49
Percentage	28.6%	22.8%	20.7%	23.4%	25.5%	17.0%

(*) indicates all plants replaced because of “sudden death”.

Plant weights

Neither of the plant weights was affected by nematode density. Therefore, regression analysis and yield modeling was unnecessary. Per variety, fresh root weights (FRW), fresh shoot weights (FSW) and fresh total weights (FTW), at all P_i values were pooled and their averages and variances estimated. The variances of the plant weight were not always the same. Therefore, pairwise t -tests were done without pooling of standard deviations. Most prominent were higher FRW of Doublet and Terranova, compared to Radical, resulting in smaller shoot/root weight ratios. Although var. Anaconda, Contra and Defender had smaller FSW and TFW than Radical, this had no substantial effect on the shoot/root weight ratio. The details of the FRW, FSW, TFW and S/R ratio of the fodder radish varieties can be found in Table 6.2, where the average weight and results of the t -tests are summarized.

Table 6.2. Pairwise t -test, of the fresh root weight (FRW), fresh shoot weight (FSW), total fresh weight (TFW) and shoot to root weight ratio (S/R) of the resistant fodder radish varieties compared to Radical.

Variety	FRW	P	FSW	P	TFW	P	S/R	P
Anaconda	15.17	0.749	186.26	* < 0.001	201.43	* < 0.001	12.28	0.055
Contra	13.14	0.118	177.88	* < 0.001	191.02	* < 0.001	13.54	0.749
Defender	16.48	0.191	187.79	* < 0.001	204.27	* < 0.001	11.40	0.827
Doublet	18.42	*0.002	215.87	0.766	234.29	0.399	11.72	* < 0.001
Terranova	20.76	* < 0.001	198.68	*0.011	219.44	0.129	9.57	* < 0.001
Radical	14.83		214.10		228.93		14.44	

(*) = significantly different from Radical.

Presence of galls

The relation between P_i and the relative percentage of plants with galled roots was also described by Equation (1) and presented in Figure 6.1. As the detection of galls is biased by the number of galls on the roots, only the parameter estimates at high P_i -values of M_{galls} were used for further analysis. Only Anaconda, Defender and Terranova had fewer roots with galls than Radical did. When M_{galls} on Radical is set to 100%, rM_{galls} on Anaconda, Defender and Terranova were 41, 43 and 28%, respectively (Table 6.3). The rM_{galls} of var. Contra and Doublet were estimated to be 71 and 68%, but due to the given coefficients of variation, varying between 0.21 and 0.59, these values did not differ materially from Radical. The results of the regression analysis of the data are also summarized in Table 6.3.

Some fodder radish plants produced galls with no or very few J2, e.g., in Radical at $P_i = 64$, Contra at $P_i = 2$ & 32, and Terranova at $P_i = 2$ J2(g dry soil)⁻¹. On the other hand, on some plant roots that were noted free of any galls, some infective J2 were extracted in the mist chamber, e.g. Radical and Terranova at $P_i = 128$ J2(g dry soil)⁻¹.

Table 6.3. Regression output of fodder radish plants with galled roots (Equation (1); see text) against initial population densities (P_i) of *Meloidogyne chitwoodi*. Where: N (number of observation), a_{galls} (maximum production of plants with galled roots), M_{galls} (maximum number of plants with galled roots), SE (standard error), df (degrees of freedom), R^2 (coefficient of determination) and the relative susceptibility of plants with galled roots (rM_{galls}).

Variety	N	a_{galls}	M_{galls}	SE_a	SE_M	df	R^2	rM_{galls}	rM_{galls} (%)
Anaconda	9	4.20	20.00	1.70	6.20	7.00	0.65	0.41	41
Contra	7	2.60	35.00	1.20	20.40	5.00	0.60	0.71	71
Defender	8	10.10	21.00	8.80	8.30	6.00	0.26	0.43	43
Doublet	9	9.10	33.00	2.70	7.00	7.00	0.76	0.68	68
Terranova	9	22.60	14.00	22.00	4.90	7.00	0.09	0.28	28
Radical	11	48.90	49.00	39.90	23.40	9.00	0.22	1.00	100

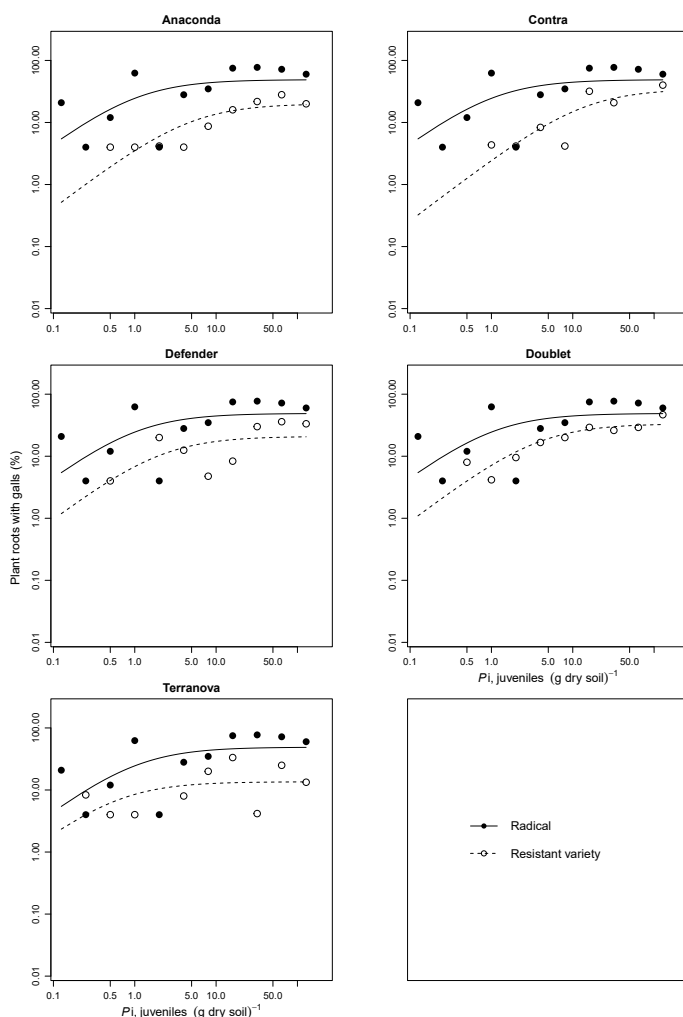


Figure 6.1. Relation according to Equation (1) between the percentage of fodder radish plants with galled roots and the initial population density of *Meloidogyne chitwoodi* of the resistant and the standard variety Radical.

Population dynamics

Final population density (P_f)

Equation (1) fitted well to the data of var. Radical and Defender but less to P_f observation of Anaconda, Contra, Doublet and Terranova (Figures 6.2 and 6.3). For Contra no estimate of a could be made. The nematode counts at higher P_i values were larger, and for all varieties, except Anaconda M could be estimated with coefficients of variation smaller than 0.5.

The population dynamics curve of the standard variety, Radical (Figure 6.2) and its 95% confidence interval, lie below the equilibrium line ($P_i = P_f$). The average maximum multiplication rate (a) and maximum population density (M) of Radical were estimated to be 0.38 and 6.43 J2(g dry soil)⁻¹ respectively. The results of the regression analysis of the population dynamic model and their 95% confidence intervals are summarized in Table 6.4. In Figure 6.3, the back transformed means of the log-transformed observations and the fitted models of the resistant varieties are compared with Radical. Despite the large coefficients of variation, the estimated population dynamics parameters of all partially resistant varieties were smaller than those of Radical. The details of the LSD-test, performed on the log-transformed parameter values, are presented in Table 6.5.

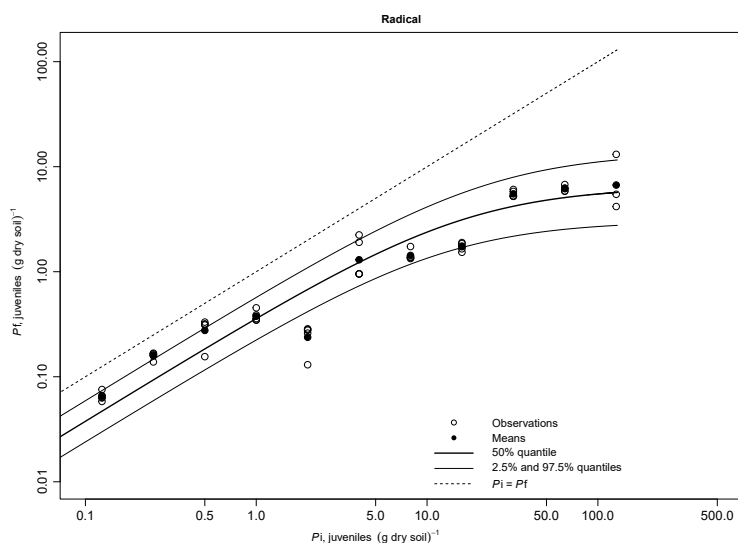


Figure 6.2. The relation between P_i and P_f of *Meloidogyne chitwoodi* of the standard variety Radical (Equation (1)). Observations (○); means (●); bold solid line: 50% quantile; thin lines: upper and lower quantiles; dotted line: equilibrium line ($P_i = P_f$).

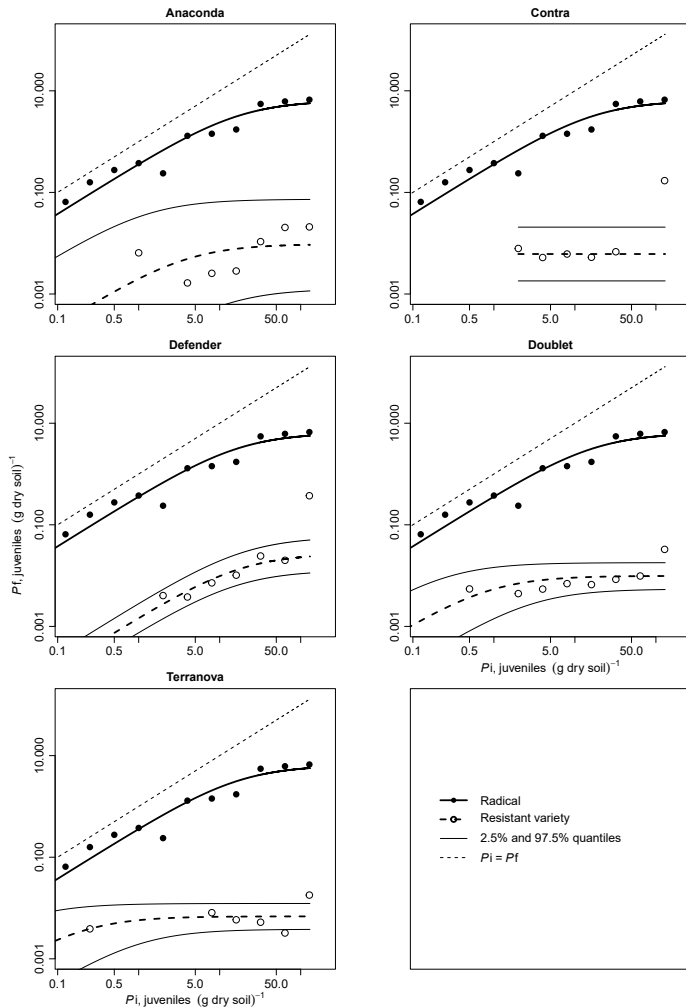


Figure 6.3. The relation between P_i and P_f of *Meloidogyne chitwoodi* of the partially resistant varieties compared to that of the standard variety Radical (Equation (1)). Dotted line: equilibrium density ($P_i = P_f$); mean P_f values of Radical (●); bold solid line: 50% quantile of Radical; mean P_f values of resistant variety (○); large dotted line: 50% quantile of the resistant variety; thin lines: upper and lower quantiles of the resistant variety.

Table 6.4. Regression output of estimated parameters of the population dynamic model (Equation (1); see text) of fodder radish varieties infected with *Meloidogyne chitwoodi*. Where: N = number of observation, a = maximum multiplication rate, M = maximum population density, J_2 (g dry soil)⁻¹, df = degrees of freedom, R^2 = coefficient of determination, CV = coefficient of variation, and Q_{2.5} and Q_{97.5} = the lower and upper quantiles of the 95% confidence interval.

Variety	N	a	M	df	R^2	cv _{a}	cv _{M}	Q _{2.5} a	Q _{97.5} a	Q _{2.5} M	Q _{97.5} M
Anaconda	7	0.002	0.011	5	-0.08	3.2	1.5	0.00009	0.045	0.001	0.1
Contra	5	NA	0.006	3	NA	NA	0.7	NA	NA	0.002	0.02
Defender	6	0.002	0.027	4	0.83	0.3	0.4	0.0008	0.003	0.013	0.06
Doublet	8	0.009	0.02	6	0.19	1	0.5	0.0017	0.045	0.008	0.05
Terranova	6	0.028	0.009	4	0.07	1.2	0.3	0.0043	0.177	0.005	0.02
Radical	11	0.379	6.429	9	0.9	0.2	0.4	0.24147	0.594	3.031	13.64

NA = not available.

Table 6.5. Comparing parameter values of the population dynamic model (Equation (1); see text) of fodder radish varieties infected with *Meloidogyne chitwoodi*. Where: N = number of observations; df = degrees of freedom; C-value = critical value; $D \log a$ and $D \log M$ = difference in log transformed parameter values of the partially resistant varieties compared with Radical; LSD = Least Significant Difference; RS = relative susceptibilities; Q_{2.5} and Q_{97.5} = the lower and upper quantiles of the 95% confidence interval; NA, not available.

Variety	N	df	C-value	$D \log M$	$D \log a$	LSD $\log a$	LSD $\log M$	RS _{a} (%)	RSM (%)	Q _{2.5} RS _{a}	Q _{97.5} RS _{a}	Q _{2.5} RSM	Q _{97.5} RSM
Anaconda	7	14	2.1	6.4	5.2	2.02*	1.55*	0.54	0.17	0.02	11.87	0.018	1.62
Contra	5	12	2.2	6.8	NA	NA	0.97*	NA	0.10	NA	NA	0.027	0.42
Defender	6	13	2.2	5.5	5.5	0.56*	0.82*	0.40	0.42	0.199	0.88	0.149	1.18
Doublet	8	15	2.1	5.8	3.8	1.17*	0.86*	2.31	0.32	0.44	12.28	0.104	0.97
Terranova	6	13	2.2	6.5	2.6	1.18*	0.76*	7.27	0.14	1.11	47.73	0.055	0.35
Radical	11	9	--	--	--	--	--	100	100	63.86	157.06	47.21	210.74

* Significantly different value from Radical.

Variation in P_i observations and the model

Mean and standard deviation were calculated for D -values at $P_i > 2$ and using these parameters random numbers were drawn from a normal distribution $N(0, 1.16)$. In Figure 6.4, the probability distribution of D is compared with that of $N(0, 1.16)$. Most marked differences are the higher frequencies near the mean (0) and the smaller frequencies at the lower tails of the distribution.

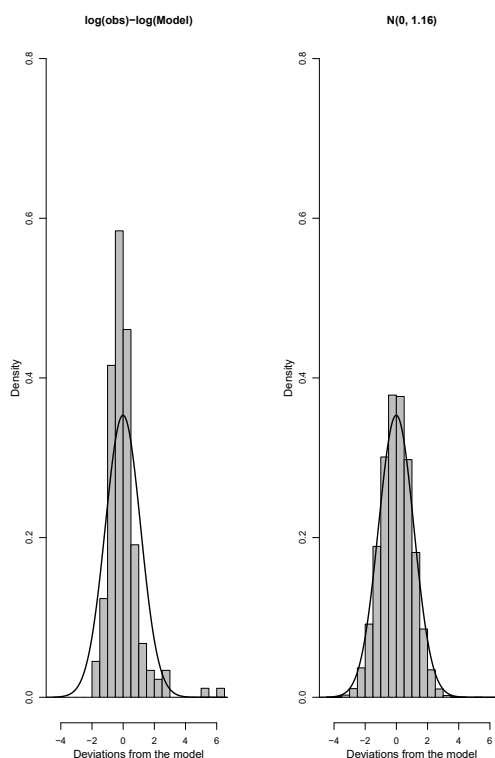


Figure 6.4. The probability distribution of the difference between the P_i of the observations of *Meloidogyne chitwoodi* on fodder radish varieties and the P_i according to Equation (1) (left), compared with standard normal distribution with the same parameters (right).

Relative susceptibility

The relative susceptibility of the resistant varieties was estimated by using Equation 2 resulting in RSa and RSM . The RSa and RSM estimate for Radical were, by definition, 100%. The RSa of the resistant varieties were not always equal to RSM , except for Defender, but due to the large variance in the parameter (a) these differences could not be marked as significant at ($P = 0.05$). In Figure 6.3, the averages of the observations and the fitted models of the resistant varieties are compared with Radical. Both the 95% confidence intervals and the small relative susceptibility, (RSa and RSM) values in Table 6.5 emphasize that the differences in relative host status between Radical and the resistant varieties was large; the averages of RSa and RSM varied between 0.4 and 7.3 and 0.1 and 0.42%, respectively.

The coefficients of variation (CV) of RSa were acceptable for Radical (0.3) and Defender (0.2), but not for Anaconda (3.2), Terranova (1.2) and Doublet (0.97). Coefficients of variation for RSM were between 0.3 and 0.5 for all varieties, except Anaconda (1.5).

In Table 6.6, the RSa and RSM values were compared to P_f/P_i ratios at $P_i = 4, 8, 16$ and $32 \text{ J2(g dry soil)}^{-1}$. The P_f/P_i values of the resistant varieties declined (P_i dependent), except for

Defender which remained the same at $4 \leq P_i \leq 64$ values. The P_f/P_i ratios at $P_i = 8, 16$ and 32 lay within the estimated 95% confidence limits of the RSM values as shown in Table 6.5.

Table 6.6. Comparing the fitted relative susceptibilities (RSa and RSM) with ' P_f/P_i ' values at P_i values of 4, 8, 16 and $32 \text{ J2(g dry soil)}^{-1}$.

Variety	RSa (%)	RSM (%)	$P_f/P_{i,4}$	$P_f/P_{i,8}$	$P_f/P_{i,16}$	$P_f/P_{i,32}$
Anaconda	0.54	0.17	0.38	0.31	0.26	0.22
Contra	NA	0.10	0.56	0.33	0.22	0.16
Defender	0.40	0.42	0.41	0.41	0.41	0.41
Doublet	2.31	0.32	1.05	0.77	0.57	0.45
Terranova	7.27	0.14	0.67	0.42	0.28	0.21
Radical	100	100	100	100	100	100

NA = not available

Discussion

Plant emergence sudden death and plant weight

A few seedlings at $P_i = 32$ and 64 and all seedlings at the highest density $P_i = 128 \text{ J2(g dry soil)}^{-1}$ died one week after sowing and emergence. This effect was also observed in field experiments with fodder radish, also only at the highest nematode densities encountered (Molendijk, pers comm.). Dead plants were replaced by 1 week old seedlings and thus exposed later to the nematode inoculum than other plants. This can have a positive effect on the minimum yield (m), (Seinhorst, 1995) and therefore on population dynamics, but all varieties were fully tolerant, with relative minimum plant weights of $m = 1$. To detect any side effects, all regression analyses were done twice: with and without the highest P_i -values. This did not make a substantial difference for the parameter estimates.

Quite a number of authors describe the observed phenomenon as 'replant disease', 'sick soil syndrome' or 'sudden death'. It is well known to occur in woody crops, e.g. apple orchards (Hoestra, 1968) or rose nurseries (Oostenbrink & Hoestra, 1961) and mostly when fields are replanted with young seedlings after removal of the old plants when nematode densities are too high to be tolerated by the young seedlings.

Meloidogyne chitwoodi is supposed to affect, in most crops, only the quality of subterranean plant parts, not the quantity (Wesemael & Moens, 2008). However, in this study the nematode inoculum was added one to six weeks after planting and therefore these estimates may be biased (Seinhorst, 1995). In a recent study, which included sufficiently high initial nematode densities, up to $256 \text{ J2(g dry soil)}^{-1}$, to estimate tolerance, *M. chitwoodi* decreased fresh tuber weight and total fresh weight (TFW) of potato cv. Desiree, until a relative minimum yield (m), of 0.86 and 0.72 respectively (Norshie *et al.*, 2011). Although in our experiment, no effect of P_i on the total fresh weight, shoot or root weight was assessed, farmers have to take into account that high densities of *M. chitwoodi* may influence plant weights negatively. Compared to Radical, the varieties Anaconda, Contra and Defender

yielded 11 - 17% less biomass (TFW), while Doublet and Terranova had 24 and 40% higher fresh root weight (FRW), respectively. These differences seem high and may have an impact on the overall performance of these varieties as a green manure. However, more pot and field experiments are needed to establish the true differences in biomass of the new varieties and their variation.

Population Dynamics

In this experiment, Radical, the “susceptible” standard variety, proved to be a bad host with multiplication rates between 0.05 ($P_i \rightarrow \infty$) and 0.38 ($P_i \rightarrow 0$). This implies that Radical reduced *M. chitwoodi* numbers by 64-95%. These results correspond with earlier findings of Al-Rehiyani & Hafez (1998b) in micro plot experiments (at $P_i = 3 \text{ J2 (ml soil)}^{-1}$ with the varieties Trez and Melodie. A multiplications rates of 0.1 were found, but not with their greenhouse experiments at $P_i = 10 \text{ (ml soil)}^{-1}$, illustrating once more, that multiplication rates are variable and that estimates in greenhouse tests are not representative for the field. Therefore, it is imperative that the population dynamics of *M. chitwoodi* on Radical is tested repeatedly under field conditions, to establish its distribution function.

The resistant varieties have shown low degrees of RS (Table 6.5) but cannot be regarded as non-hosts because their P_f values are P_i dependent; new nematodes were formed, albeit in much lower numbers than the initial population. If the reproduction of *M. chitwoodi* under field conditions on Radical is the same or smaller than in this experiment, the partially resistant varieties reduce *M. chitwoodi* infestations within 11 weeks by 98% or more. This decrease adds up to the so-called ‘natural decline’ of *M. chitwoodi* during winter. This winter decline was monitored on 4 fields in Belgium and The Netherlands and estimated to be 5.5% per week according to an exponential model (Been *et al.*, 2007). This model implies that decline is independent of time and nematode age. During the period from September until May next year (36 weeks), this decline would normally be 86%. With fodder radish during the first eleven weeks and a further population decrease of 74% during the next 25 weeks a decline of at least 99.8% would be obtained - all under the given assumptions. This seems a lot, but unfortunately, the P_i threshold of *M. chitwoodi* for quality damage is extremely small. In a greenhouse test with the potato cv. Desiree it was smaller than $0.0625 \text{ J2(g dry soil)}^{-1}$, the smallest density in the test. At $P_i = 0.5 \text{ J2(g dry soil)}^{-1}$ 60% of the tubers were infested (Norshie *et al.*, 2011). To reduce nematode infestations to that level, the original population density at planting time of fodder radish – early September - must be equal to or smaller than $ca 60 \text{ J2(g dry soil)}^{-1}$, still supposing that the population dynamics of *M. chitwoodi* on Radical is the same or smaller under field conditions than it was in the greenhouse. For the new resistant potato genotypes (Norshie *et al.*, 2011) the combination of potato after fodder radish looks more promising.

Deviations of P_i observations and models

Estimation of the probability distribution of the difference between the P_i observations and model values is important step in the screening of seeds like fodder radish. Using Monte Carlo simulations, it will allow the estimation of the number of observations needed to estimate host-status with predefined precision. In this experiment, variations are high at low densities ($P_i \leq 2$) due to low nematode counts as compared to higher densities ($P_i > 2$). Besides, in resistant plants like fodder radish with a very low RS, zero values of P_i either from the roots or soil are common at low P_i values. These zeros are not considered as 'true' zeros, only the result of too small subsamples. Therefore, zeros were replaced by half of the minimum within an observation, but this procedure underestimates the variances at small densities. In further research, larger subsamples will be needed to count at least 100 nematodes per sample. If not, the P_i variation will be highly dependent on laboratory procedures and not on the "true" variation of the seeds. If possible, in future, routine testing at medium P_i values is preferred compared to the time consuming, small P_i values. Densities $< 32 \text{ J2(g dry soil)}^{-1}$ are suggested to avoid the risk of "sudden death".

Estimators of degree of resistance in varieties of a crop

Mainly because of the many zero counts at small P_i values ≤ 2 and the small multiplication rates, the estimates of parameter a were uncertain, which was reflected by the high coefficients of variation of this parameter for all varieties, except Defender and Radical. Radical and Defender had the same M/a ratios, indicating that for Defender RS is the same at all P_i values. For the other tested varieties, it is uncertain whether or not $RSa = RSM$. The relatively large variation of the parameter a and the resulting small values of R^2 do not allow further comparisons. Therefore, more research is needed to develop simplified routine tests for partially resistant varieties.

If the measured RS value is not P_i independent, other options can be pursued. Testing at more than one P_i would make it possible to estimate both RSa and RSM . If a stable relation between both estimators were reported, again a single estimator would suffice. Another possibility is to focus on the RSM . Because of the high tolerance ($m = 1$) of the fodder radish varieties, P_i and RSM are not P_i dependent from a certain point on where (M) is reached. Population densities of *M. chitwoodi*, at the time of sowing of a resistant fodder radish variety, will predominantly be higher than the (M) value of the fodder radish variety. In that case, population densities will decline to (M) of the resistant fodder radish variety and estimation of the RSM then suffices to quantify the effect of these varieties.

The fact that total fresh weight is barely or not affected by *M. chitwoodi* is favourable for the development of a routine test at relatively higher nematode densities, than are usual for other crop/nematode combinations. For potato cyst nematodes a P_i of $5 \text{ J2(g dry soil)}^{-1}$ is chosen in routine tests to avoid both small nematode counts and large variations at small P_i values and decreased plant weights and P_i values at high P_i values.

In this experiment the relative susceptibility (rM_{galls}), based on the percentage of galled plants, does not provide sufficient information on the degree of resistance (RSM). No distinction could be made for instance between Radical, Doublet and Contra or Anaconda, Defender and Terranova based on rM_{galls} . Several reports in the literature indicate that root gall index and/or presence or absence of galls was not always correlated with nematode reproduction (Holbrook *et al.*, 1983; Araya & Caswell-Chen, 1994; Al-Rehiyani & Hafez, 1998b). Therefore, presence or absence of galls or root gall index may be used during a bulk-screening program by plant breeders when screening many varieties at the same time, because of its simplicity and cost effectiveness. However, at the end stage of certifying and releasing a variety, resistance has to be described as RS based on a population dynamic model and the use of proper P_i values.

Conclusion and recommendations

The results obtained in this experiment show that the tested fodder radish varieties have a high resistance and, therefore, should be efficient in reducing population densities of *M. chitwoodi*.

Initial density independence would open the possibility to develop an easy and cheap partial resistance test comparable to the one developed for potatoes and potato cyst nematodes. As indicated, more information is required about the stability of RSa and RSM. If both are equal, it is theoretically possible to test at a single population density, as the P_i does not affect the RS. In a follow-up experiment, more care has to be taken to obtain larger nematode counts at low P_i densities by either investigating the whole suspension or elutriating a second subsample of soil, thereby avoiding zeroes or low P_i counts.

Another point of interest in developing a resistance test is the variability of the resistance throughout the seed lot. The measured resistance could be the result of a mixture of fodder radish seeds, which are either resistant or susceptible, or it could be that each seed has a different degree of resistance.

Relative susceptibility, RS values for resistant fodder radish varieties can be integrated in a Decision Support System (DSS) like NemaDecide (Been *et al.*, 2005) to predict both population development and calculate possible yield losses in following crops. To use this information to its fullest, minimum threshold values for quality damage of economically important crops, *e.g.* potatoes, black salsify or carrots are required. Current information indicates that the threshold values for these crops are very small. As threshold values are difficult to establish in the field, due to sampling errors, availability of the required range of population densities, presence of other pathogens and the general variability encountered in field experiments (Hussey & Boerma, 1981), this research should be undertaken in the glasshouse under controlled conditions.

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Chapter 7

General discussion

Introduction

The restrictions imposed on the use of chemical control of plant-parasitic nematodes prompted the need for alternative management strategies for *Meloidogyne chitwoodi* (Duncan, 1991). Conventional rotation with non-hosts was the first strategy that was pursued. However, only a few non-hosts of *M. chitwoodi* were found (www.aaltjesschema.nl). Consequently, research was initiated to screen for resistance genes and breeding programs were started (Zoon *et al.*, 2002). The availability of a methodology for estimating either host-status of susceptible crops or the degree of resistance is crucial in using poor hosts and resistant crops in rotation schemes to manage the pest. The part of the research published in this thesis focuses on evaluating the resistance of potatoes and green manure crops. It started with the development of a standard methodology for estimating resistance, assessing yield and quality losses (potato tubers) using relevant models. In total 14 potato genotypes and 9 fodder radish varieties with resistance to *M. chitwoodi* were evaluated for their resistance, yield and quality loss, under glasshouse and field conditions. In this chapter, a general discussion of the results and possible implications are presented.

A reliable routine resistance test

The availability of a reliable method for the estimation of resistance was necessary after recognition that some potato cultivars, with resistance against *Globodera pallida*, did not show the complete resistance as those potato cultivars with the H_1 -resistance gene against *G. rostochiensis*, but a spectrum of resistances between 0 and 100%. It was also necessary for the practical utilization of these resistant cultivars by farmers using an advisory system (Been & Schomaker, 2004; Been *et al.*, 2005). The concept of relative susceptibility (RS) (Chapter 1) was adapted in The Netherlands for controlling PCN in 2000 and by the EU member states since 2006 after it was reported in 1984 by both Phillips and Seinhorst, separately. Because of the introduction of the methodology and the resulting availability of potatoes resistant to PCN (Seinhorst, 1984; Seinhorst *et al.*, 1995), this nematode is under control in The Netherlands.

The concept of RS is now extended to *M. chitwoodi* resistant potatoes (Chapter 2) and fodder radish (Chapter 6). All tested genotypes proved to be highly resistant ($RS < 1\%$). The population dynamic curves of *M. chitwoodi* of the susceptible reference potato cv. Desiree and 6 out of 7 tested resistant genotypes run parallel to each other (Chapter 2), despite the different sources of resistance used.

The parallel lines over the range of P_i values in (Chapter 2) indicate that the M/a ratio of the susceptible and the resistant host are the same and that $RSa = RSM$. If the population dynamics lines run parallel, then RS is P_i independent. This enables the possibility to estimate RS at a single well-chosen P_i and a standard testing method for the breeders now becomes feasible. As a pot size of 10 kg, used to provide the one stem potato with an equivalent amount of soil and inoculum as in the field, is not a very handy unit for routine testing, so it was necessary to verify whether downsizing the pot size used for a standard resistance test will not interfere with the reliability of the RS estimator. If roots get pot bounded as a result

of limited space in the pot, the nematode inoculum will be depleted while the roots still are growing; the new roots will not be invaded and the model parameters (Chapter 1), the maximum multiplication rate (a) and the maximum population density (M) will change (Seinhorst, 1979). As both the susceptible and the resistant host are tested under the same conditions this might not be a problem, but must be checked. In Chapter 2, this is investigated when downscaling the pot size from field level conditions to a 2 kg pot, which is simple enough to be handled by the breeders and laboratories. It was demonstrated that, although the parameters did change, the resulting RS remained the same for *M. chitwoodi*. The described methodology is expected to provide for a generic assessment of resistance of crops to plant-parasitic nematodes. The generated resistance parameters (Chapter 2) can be incorporated into a decision support system (DSS) e.g. NemaDecide, to help extension services and growers to explore the use of resistant cultivars. This includes their effect on population development, yield and quality losses and ultimately calculating the cost benefit of scenarios to manage *M. chitwoodi* infestations. Apart from describing the host suitability of every nematode/plant combination and estimating the degree of resistance, the same parameters and models (Seinhorst, 1966, 1967, 1970) can be used to address other challenges which were also part of the objectives of the DREAM project (Chapter 1) and others e.g.:

- i) To detect differences in virulence between different population of the same nematode species.
- ii) To detect genotypes with deviating M/a ratios as in 2011M1 (Chapter 2).
- iii) To carry out cost/benefit analysis for those control measures which influence the population density and are used in integrated management strategies.

When RSa does not equal to RSM the population dynamic lines will not run parallel (Figure 7.1 A and B), the M/a ratio then is different between both cultivars, and resistance cannot be measured at a single P_i . If this condition applies we need to test resistance at two different densities if possible; one at lower P_i and the second at a higher P_i to obtain estimates of both a and M .

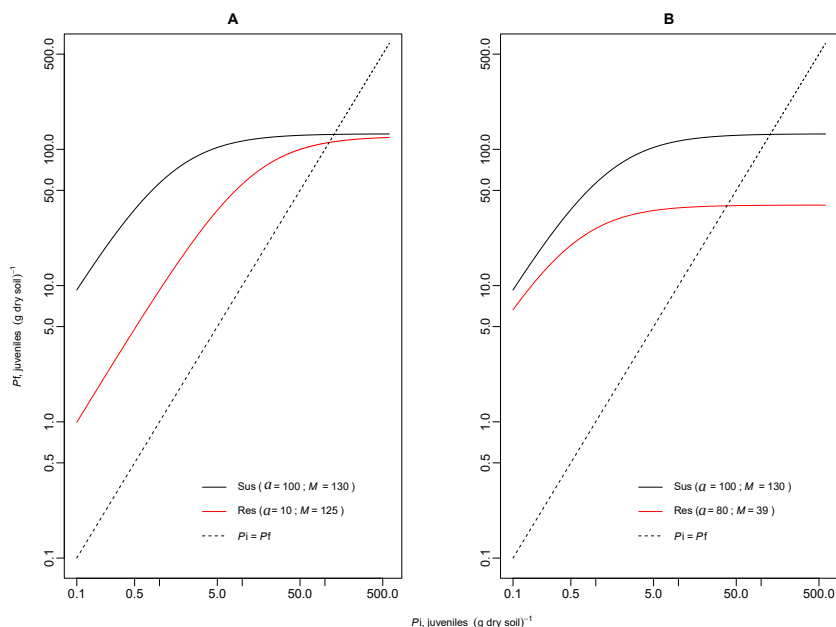


Figure 7.1. Population dynamics lines according to Equation: $P_f = M \times P_i / (P_i + M/a)$ depicting the relationship between P_i and P_f (log scale) of *Meloidogyne chitwoodi*: Two situations where the P_f lines are not parallel: $M/a_{\text{sus}} \neq M/a_{\text{res}}$. **A)** Parameter M of the susceptible and the resistant host are the same. Now $RSa = 10\%$ and $RSM = 96\%$. **B).** Parameter a of the susceptible and the resistant host are almost similar and M of the resistant host is lower, $RSa = 80\%$ and $RSM = 30\%$. In Both **A** and **B**, RS is density dependent. Solid black and red lines are the susceptible and resistant hosts respectively, diagonal broken line is the equilibrium density where $P_i = P_f$.

Minimum yield (m)

At very high nematode densities plant size is decreased by nematode attack; yield, haulm weight and root weight are smaller compared to a plant not attacked by nematodes. When the root size decreases, also, the available space for nematodes inside the roots is reduced and as a result, population densities will be smaller than the maximum population density M of a more 'tolerant' crop. If the susceptible or the resistant host is intolerant above a certain P_i , resistance can be either underestimated (Figure 7.2 B) or overestimated (Figure 7.2 C). When both the tested genotype and the control cultivar are intolerant but have the same minimum yield (m), RS remains independent of P_i (Figure 7.2 D).

The latter occurrence is unlikely. More likely, both cultivars will have different m values, the lines will not be parallel over the whole range of P_i values, and the resistance estimator might be affected when a single P_i is used for the standard resistance test. In those situations care has to be taken at which single P_i resistance has to be tested; P_i 's above the density where plant growth is affected should be avoided. Affected plant growth was observed with cv. Desiree in all four experiments and three of the tested genotypes (Chapter 2). Hence, a test $P_i = 24 \text{ J2(g dry soil)}^{-1}$ was proposed for the routine test, where this effect does not interfere with the estimation of the RS.

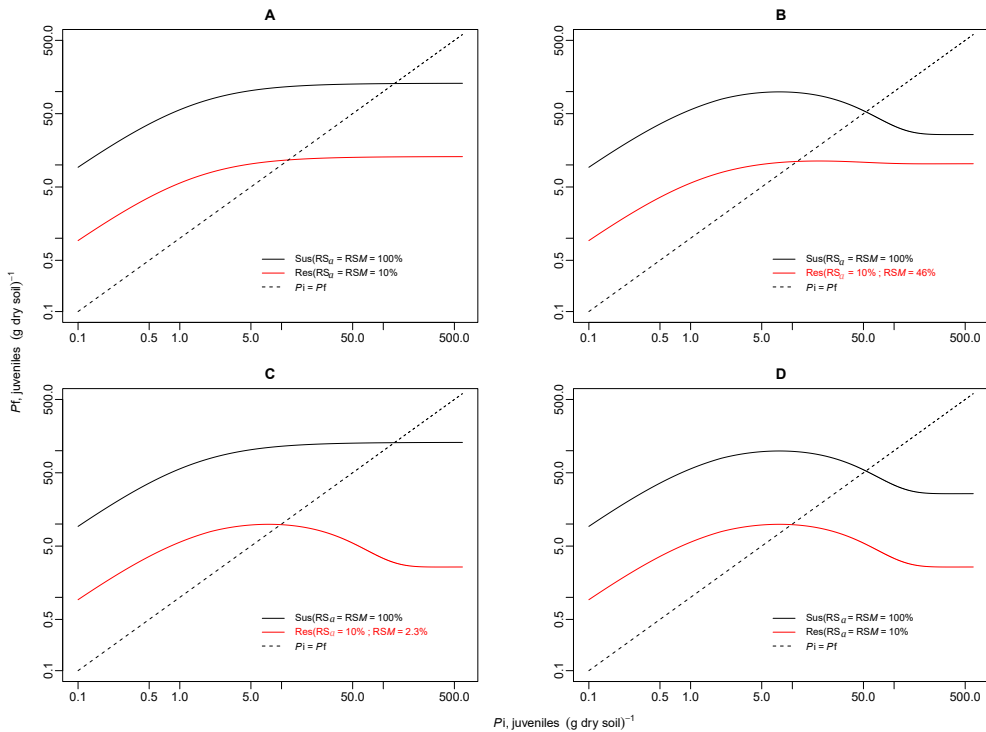


Figure 7.2. Population dynamics lines (log scale) according to Equation: $P_f = y \times M \times P_i / (P_i + M/a)$ describing the relation between P_i and P_f of *Meloidogyne chitwoodi*. The population dynamic model is extended when intolerance is observed using yield loss model (Seinhorst, 1965): $y = m + (1-m)0.95^{P_i/T-1}$. **A)** Both the susceptible and the resistant host are tolerant ($m = 1$) and lines are parallel, $RS = 10\%$. **B)** Susceptible host (black line) is intolerant ($m = 0.2$). Estimating RS at a single $P_i > 100$, will result in an overestimation of $RS = 46\%$. **C)** Resistant host (red line) is intolerant ($m = 0.2$). Estimating RS at a single $P_i > 100$, will result in an underestimation of $RS = 2.3\%$. **D)** A situation where the P_f lines are parallel and the RS is not affected as both the susceptible and the resistant host are intolerant ($m = 0.2$). Diagonal broken line is the equilibrium density where $P_i = P_f$.

Differences in root size

When the tested genotype and the control are of different plant species, *e.g.* when maize and carrot are compared, their P_f lines cannot be expected to run parallel. Different crops have different root volumes that will also produce different maximum population densities (M) (Pudasaini, 2006). In addition, the suitability of roots for multiplication of the nematodes differs between crops. This implies that both population dynamic parameters (a and M) differ between crops. In fact, the population dynamic model of both crops may even intersect (Figure 7.3) which means that host suitability of the two crops becomes density dependent. At $P_i = 10 \text{ J2(g dry soil)}^{-1}$, both crops produce the same population density. At $P_i < 10 \text{ J2(g dry soil)}^{-1}$ crop 1 produces less J2 and should be preferred while at $P_i > 10 \text{ J2(g dry soil)}^{-1}$ crop 2 is better for nematode management. Therefore, host status or resistance cannot not be estimated by comparing two different crops at a single nematode density. Host status then has to be described by estimating both parameters a and M using a suitable range of P_i values.

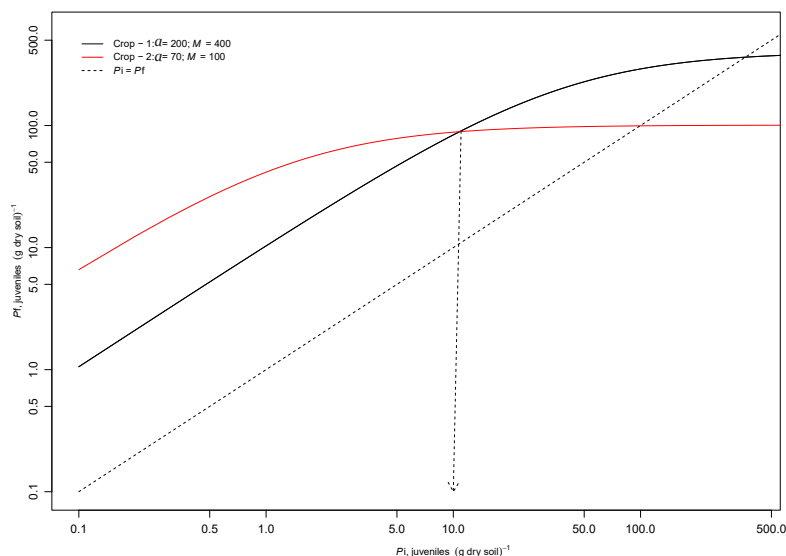


Figure 7.3. Population dynamics lines *Meloidogyne chitwoodi* according to Equation: $P_f = M \times P_i / (P_i + M / a)$, describing the relation between P_i and P_f (log scale) of two different crops. Crop 1 has a higher maximum multiplication rate (a) but reaches a smaller maximum population density (M). Crop 2 has a lower multiplication rate (a) but reaches a higher maximum population density (M). Both lines are intersecting at $P_i = 10 \text{ J}_2(\text{g dry soil})^{-1}$. Diagonal broken line is the equilibrium density where $P_i = P_f$.

Fodder radish resistance

All tested fodder radish varieties proved to be highly resistant ($RS < 1\%$). The question if there is P_i independence of the RS estimator ($RSa = RSM$) cannot be confirmed statistically. This is due to the high resistance of the tested fodder radish varieties resulting in such a low numbers of J_2 in the P_i that estimation of the parameter a became highly unreliable. The susceptible fodder radish variety Radical proved to be a poor host (Chapter 6). Each P_i produced a P_f that was lower than the P_i . In a follow up experiment (data not published), this was again confirmed and it was demonstrated that a second susceptible control, Siletina, also proved to be a very poor host (Chapter 1. Figure 1.5 A).

A management system for *Meloidogyne chitwoodi*

Resistant main crops, green manure crops and natural decline

As was indicated in Chapter 1, despite quarantine regulations, new *M. chitwoodi* infections are found in The Netherlands every year. If unchecked, this nematode will ultimately limit the area where potato seed can be produced and cause severe economic losses. In countries like The Netherlands with high cropping frequencies of potato, managing this nematode and securing arable land for seed production is of prime importance.

An integrated approach that includes adjusting the sowing date, using poor hosts, resistant green manure crops, black fallow and nematicides, was advised in The Netherlands (Molendijk & Korthals, 2005). A similar approach was also recommended to manage *M. chitwoodi* in the US (Strand, 2006). Considering some of the bottlenecks mentioned in Chapter 1 concerning the management options for *M. chitwoodi*, the availability of resistant potato genotypes and fodder radish varieties will prove to be valuable additions in the management of this nematode.

An example of a management scenario (from spring to spring)

A large reduction of the population density can be expected when either resistant potato genotypes or fodder radish varieties are grown. Let us assume growing a resistant potato crop from mid-April to early September and fodder radish from late September to mid-November (Figure 7.4). According to Been *et al.* (2007) the natural decline of *M. chitwoodi* under field situations is about 5.5% per week independent of nematode age. The maximum field infestation so far encountered in The Netherlands is $5 \text{ J}_2(\text{g dry soil})^{-1}$ in spring. Let us double the population density in spring, $10 \text{ J}_2(\text{g dry soil})^{-1}$, a worst-case scenario. The cropping of a resistant potato cultivar, *e.g.* AR04-4096, will result in a reduction of approximately 98% of the population to *ca.* $0.2 \text{ J}_2(\text{g dry soil})^{-1}$ by the first week of September. A subsequent resistant fodder radish crop, *e.g.* Contra, will further reduce the population density to approximately $0.006 \text{ J}_2(\text{g dry soil})^{-1}$. A natural decline for 25 weeks should reduce the population before the next spring by another 74%, which will bring the population theoretically down to $0.001 \text{ J}_2(\text{g dry soil})^{-1}$. We have now created a very low P_i for a next crop.

Already after cropping only a resistant fodder radish variety at a P_i of $10 \text{ J}_2(\text{g dry soil})^{-1}$ no quality damage should be observed when a resistant potato genotype is grown, except 2011M1 (Chapter 3). The produced tubers would be acceptable by the processing industry.

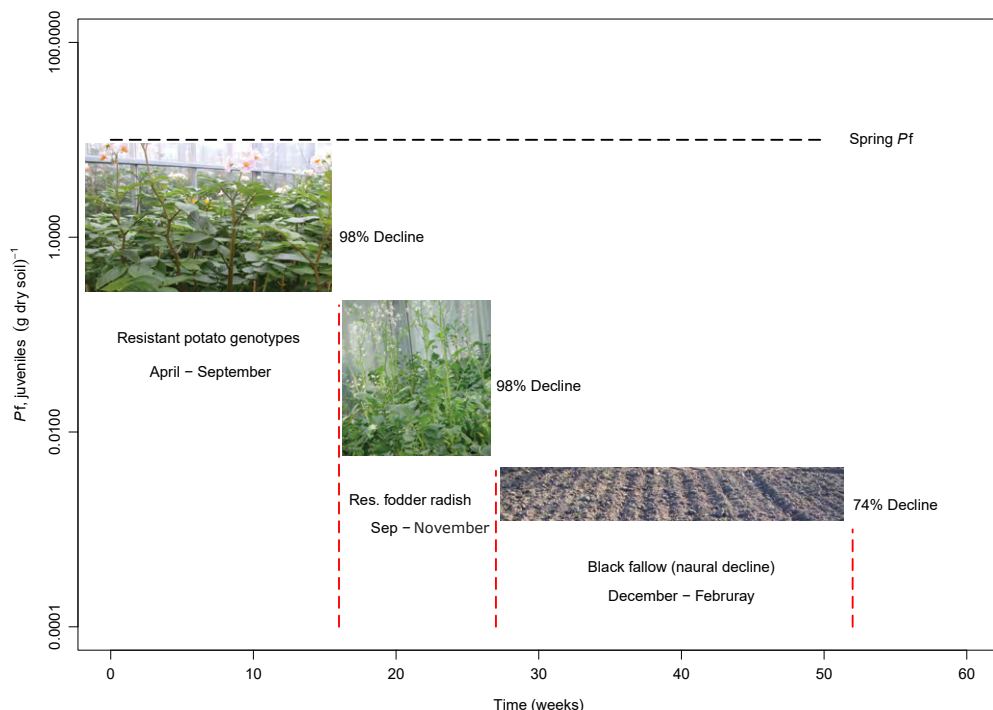


Figure 7.4. A possible rotation scheme to control *Meloidogyne chitwoodi* using resistant potatoes, fodder radish varieties and black fallow.

The effect of the resistant potato genotypes has already been tested in a pilot field test in 2015 and in a larger scale field test in 2016. In both tests the estimated resistance and quality improvement of the potato genotypes in the glasshouse tests was validated successfully in the field (Teklu *et al.*, unpubl.). A close follow up of population densities in infested fields when testing management scenarios is needed to obtain more information and to parameterize the models used for DSS's. Moreover, monitoring regularly by soil sampling helps to evaluate population development and to decide whether a field can be used for production of a valuable crop *e.g.* potato seed. As most tested potato genotypes and all tested fodder radish varieties are highly tolerant – no reduction of the root system is to be expected – a maximum effect of these resistant crops on the population density can be expected. However, care has to be taken to remove all potential hosts *e.g.* weeds that can maintain population densities of *M. chitwoodi* during fallow.

Plant growth and fresh tuber yield

All tested genotypes and susceptible controls, whether potato or fodder radish, showed a normal logistic growth in time. At the highest P_i values growth was delayed and stems were longer but thinner. In this research earlier reports on fresh tuber weight reduction caused by *M. chitwoodi* was confirmed (Pinkerton & Santo, 1986; Norshie *et al.*, 2011) for potato cultivars. Four of the seven genotypes tested were tolerant ($m = 1$) as in Figure 7.5. The observed decrease

in yield of two starch potatoes and one ware potato genotype occurred at very high P_i 's. These P_i 's will not be encountered in the field in spring and no yield loss was suffered when a selection of the tested genotypes were grown in the field validation tests (Teklu *et al.*, unpubl.).

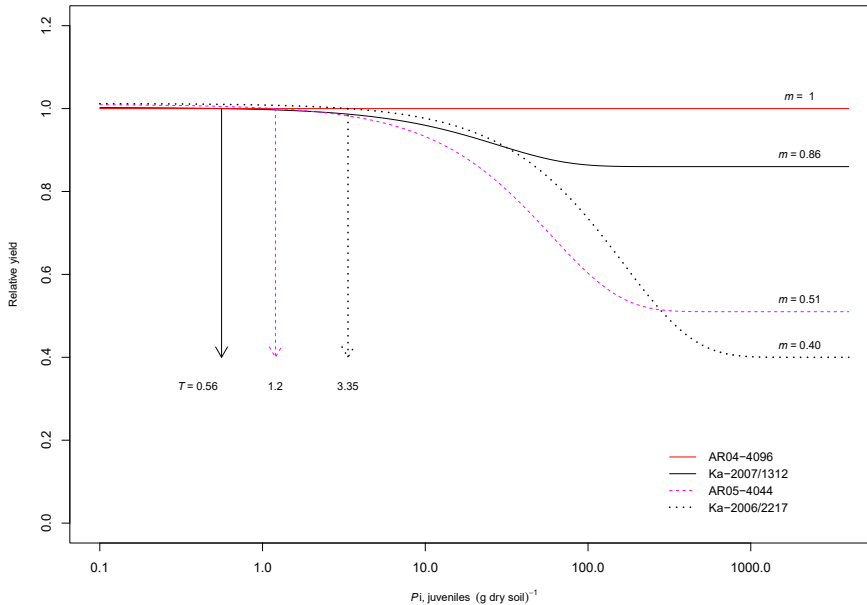


Figure 7-5. The relation between P_i (log scale) of *Meloidogyne chitwoodi* and relative fresh tuber yield (y) according to Equation: $y = m + (1 - m)0.95^{P_i/T-1}$ for potatoes with different relative minimum yields (m) and tolerance limits (T) (Seinhorst, 1965).

Quality improvement of potato tubers

Quality of tubers for processing by industries

More than yield loss, the quality of the tubers (ware potatoes) or their infestation (seed tubers) are important economic issues. In general, quality of the resistant potato genotypes was extremely improved compared to cv. Desiree (Teklu *et al.*, 2016). Nearly all genotypes with resistance are acceptable for industrial processing with a tuber knot- index (TKI) < 10, except 2011M1.

Quality of tubers as seeds

The proportion of clean tubers over all densities is > 90% for the resistant genotypes, except for genotype 2011M1, compared to less than 10% on cv. Desiree. Result from the field validation test provided similar results. Although quality was improved significantly, these tubers are still not acceptable as seed potatoes, considering the zero tolerance for *M. chitwoodi*. The risk of exporting such lots will then depend on the reliability of detection during certification by the exporting country and the import check of the importing country (EPPO, 2006).

Potato root and tuber resistance

Brown *et al.* (2009) demonstrated the independent expression of resistance in both the roots and the tubers of potato plants. In pot experiments, this might create a problem when both should be tested simultaneously. Root resistance could be estimated as done in Chapter 2, but tuber resistance might not be tested correctly as, when roots are highly resistant, infective J2 might be depleted at the time of tuber setting, and tuber resistance would be assumed erroneously.

In Chapter 4 this question is explored by providing an exogenous source of nematodes during tuber setting (Brown *et al.*, 2009). The system used in this thesis was to grow a good host, bristle oat (*Avena strigosa*), alongside the tested potato genotype (Figure 7.6). *Avena strigosa* will multiply *M. chitwoodi* and increase the level of inoculum in the presence of resistant potato roots and provide J2 during tubers setting. Although final population densities in the soil were drastically increased in the presence of *A. strigosa*, tuber resistance could still be demonstrated. Despite its significant tuber resistance, genotype 2011M1 showed a disproportional increase in quality damage in the presence of *A. strigosa*. This indicates that quality damage is not an estimate for resistance or vice versa. In addition, the need for weed control when growing these genotypes and potatoes in general, to avoid unnecessary population build up is confirmed. As the pot test was done only at a single initial density, $P_i = 16 \text{ J2(g dry soil)}^{-1}$ it would be recommendable to repeat this experiment using a full range of P_i densities to model the complete population dynamics with and without *A. strigosa*.

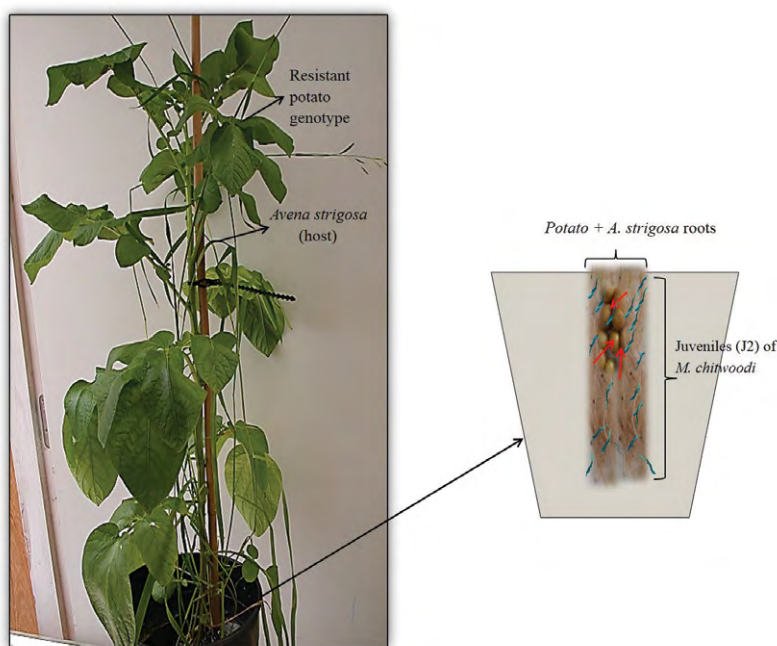


Figure 7.6. Testing the independence of root and tuber resistance using an external source of exogenous inoculum (in this case *Avena strigosa*) in pot test, partly adapted from (Brown, 2011).

Population changes of *Meloidogyne chitwoodi* in infected tubers during storage

As tuber infection is a major problem when exporting potatoes to different countries, it seems logical to explore what happens to population densities of *M. chitwoodi* in tubers during storage. In this thesis (Chapter 5), the three most frequently applied storage temperatures were used to store infected tubers of cv. Desiree for up to 240 days and population development in time was studied. *Meloidogyne chitwoodi* densities declined at 4°C and at 8°C with 91% and 65%, respectively. Information from the literature could not provide a plausible explanation for this decline, though an increased sugar level at low temperatures is suspected (Olthof & Yu, 1999). At the highest storage temperature (12°C), the nematodes increased 2.5 times directly after harvest during the first 60 days of storage and then remained constant. This was attributed to matured females, which continued to produce new eggs for a short time after storing. No multiplication occurred during storage, which was also reported by Ingham *et al.* (2000) and Powers *et al.* (2005). Surviving nematodes originating from all storage temperatures and times were vital, able to reproduce on cv. Desiree and cause heavy tuber deformations in the next crop.

According to Chapter 4, the tubers of the two resistant potato genotypes AR04-4096 and 2011M1 contain a P_{tuber} of 0.01 and 0.18 J2(g dry soil)⁻¹, respectively at $P_i = 16$ J2(g dry soil). Using the information on the population decline at 4°C during 240 days of storage for *M. chitwoodi*, these densities will be reduced to 0.00084 and 0.017 J2(g dry soil)⁻¹ for AR04-4096 and 2011M1, respectively.

Tubers of resistant genotypes infected at a range of P_i 's 0.125-128 J2(g dry soil)⁻¹ and stored at 7°C for 240-300 days were found positive for *M. chitwoodi* though the numbers were very low ca. 0.002 J2(g dry soil)⁻¹ when compared to 0.35 J2(g dry soil)⁻¹ of cv. Desiree (Chapter 2 and Chapter 3). The probability of causing quality damage at these densities can be considered minimal, but needs to be checked.

Remaining knowledge gaps and concluding remarks

Methodology

Resistance testing involves several techniques starting with culturing and inoculation, up to the extraction of J2 for quantitative analysis. Efforts in calibration and improving the efficiency (recovery and low variance of numbers) of the equipment used has been a large part of the research (Teklu *et al.*, 2013a,b). Techniques used in quantitative nematology are a decisive factors in estimating resistance with a certain degree of distinctiveness. Several methodological improvements were established, especially techniques used for the extraction of nematodes from soil, roots and tuber peel. Some examples:

- i) As whole root systems are used to estimate J2 in the organic fraction, in order to reduce the high variation in numbers when only the organic fraction from the processed soil sample is used, large amounts of roots have to be put into the mist chamber. A detailed

study was required to determine the maximum amount of organic material per cm² of extraction sieve, as too large volumes inhibited the hatching of J₂ and caused underestimation of population densities (Teklu *et al.*, 2013a). The same inhibition of hatch occurs when too high amounts of tuber peel per cm² sieve were incubated. Moreover, the starch from the dissolving peel caused extraction sieves as used for the roots to clog and larger sieve apertures were needed to avoid this problem. Blind chopping of roots or tuber peel and using extraction sieves without any knowhow of these effects does not contribute to a reliable estimation of resistance.

- ii) The final population density (P_f) of the resistant potato and fodder radish varieties is very low, particularly at the lower initial population densities (P_i) (Chapters 2, 4, 6). This caused difficulties to estimate the maximum multiplication rate (a). To be able to obtain more J₂, larger soil samples had to be processed and the capacity of the Seinhorst elutriator for extraction of nematodes from soil was upgraded from 500 to 800 g dry soil. Recovery for *M. chitwoodi* using the Seinhorst elutriator is now 92% with a coefficient of variation < 7% (Teklu *et al.*, 2013b).

Population dynamics of *Meloidogyne chitwoodi* in time

The growth period of potato in the pot test varied between 13-16 weeks. Just like the population dynamics of *P. penetrans* on several crops *e.g.* potato, maize, carrot and black salsify (Pudasaini, 2006), it might be possible that the highest population densities occur weeks before harvest. Then the number of J₂ would be higher and RS could be easier and more reliably established. Another possibility might be that the RS is influenced by the growing period when early and late varieties are compared. To research this, time series of P_f and growth of plant parts – haulm, roots and tubers – are required. Unpublished data (Figure 7.7) confirms that the maximum population density indeed is attained earlier in the growing period, around 9-10 weeks after planting, and not at harvest. A detailed analysis of these data will allow us to investigate if and how this information can be used to improve the reliability of the RS estimator.

Screening for virulence of *Meloidogyne chitwoodi* populations

Currently, the Smakt population (Mc31) from The Netherlands is used as a test population. This population is also used for research in Belgium, Germany and France. Screening populations of *M. chitwoodi* and selecting the most virulent population as a testing population is necessary for future use in a standard resistance test. Already more than 60 populations have been collected from all over The Netherlands, but also from Belgium, Germany and France. The first pilot test of this project (MeloVir) has already been started in 2017 with 20 of these populations using two resistant potato genotypes and cv. Desiree. The Smakt (Mc31) population from WUR PAGV, is included as a reference.

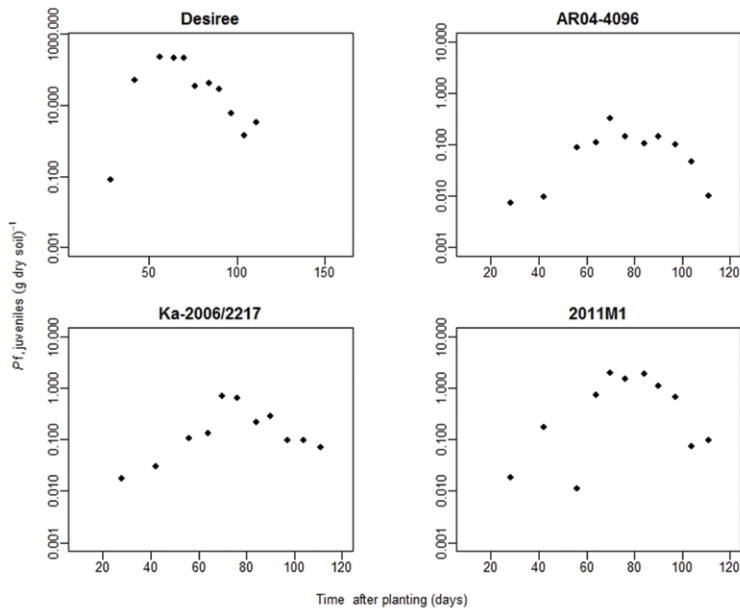


Figure. 7.7. Population development in time (complete growing period) of *Meloidogyne chitwoodi* on cv. Desiree and three tested genotypes.

Variation in resistance of fodder radish seeds

Resistant fodder radish seed is a mixture of both susceptible and resistant seeds and are all genetically different (Chapter 6), unlike the clones of potato tubers (Chapter 2). In Chapter 6, based on the seeding rate of fodder radish under field conditions 25 seeds per density were used: 5 seeds per pot in 5 replications. By using a range of P_i 's ultimately 225 seeds were tested per variety. For a stable resistance measurement at one single density, research is required to determine the exact number of seeds to be tested for each variety to provide a reliable RS estimator with an acceptable level of variation. In a follow-up experiment (Teklu *et al.*, unpubl.) extra replications at some P_i 's were used to collect the required data. A Monte Carlo simulation will be used to determine the required number of seeds to obtain a stable resistance estimator.

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Summary
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Summary

Considering its biology, host preference and the limited management options available, development of resistance is the key for the management of *M. chitwoodi*. After developing resistant crops, the degree of resistance has to be quantified. Methodology has to be developed, a test protocol devised and problems have to be addressed. The results presented in this thesis are only a small part of the research effort of the last seven years and can be considered as the major insights out of all the information that became available.

In **Chapter 2** of this thesis it was proven that genotypes, with a single resistance gene towards *M. chitwoodi*, have remarkably a high level of resistance (> 99%) and that all sources of resistance used performed equally well. Resistance, expressed as relative susceptibility (RS) proved to be P_i independent for potato and *M. chitwoodi*. As a result, the development of a simple, but reliable quantitative resistance test at a single well-chosen P_i and a small pot size became feasible. In **Chapter 3** it was demonstrated that yield losses were noticed in 3 out of 7 genotypes, but at population densities that will hardly be encountered in the field. More problematic is the quality loss due to *M. chitwoodi*. While almost all genotypes, at all initial population densities, showed a tuber knot-index value (TKI) that made them suitable for industrial processing, tubers are still infected with *M. chitwoodi*, although in small numbers, which still might cause problems in seed potatoes. In **Chapter 4** the possibility of independent inheritance of resistance in roots and potato tubers for *M. chitwoodi* was explored. This was tested on two resistant genotypes and resistance was confirmed in both plant parts. The results emphasized the need for weed control when *M. chitwoodi* is present in the field. In **Chapter 5** the post-harvest development of *M. chitwoodi* during storage at three different storage temperatures in time is presented. Different effects are encountered at the different storage temperatures used. While at lower temperatures 4°C and 8°C population densities declined, at high temperatures (12°C) the population densities remain unchanged after an initial increase. In **Chapter 6** the resistance of fodder radish varieties was tested. All tested varieties had a very high resistance (> 98%). Surprisingly, even the susceptible fodder radish varieties proved to be very bad hosts reducing population densities over the whole range of P_i 's used.

The developed methodology - a draft of the standard operating procedure (SOP) is available - and the acquired knowledge is already in use by potato breeders in The Netherlands. This is expected to facilitate breeders, work in producing more resistant and tolerant crops that can ultimately be used as strategic tools to manage *M. chitwoodi*. The information concerning the resistant genotypes and fodder radish varieties will be included in the Dutch Decision Support system NemaDecide Geo plus, which runs on the web-based smart farming platform Akkerweb to be used by farmers and extension services to control *M. chitwoodi*.

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At the end of the day, the Ph.D. is finished. I found it to be the most triggering accomplishment of my life. It is true that throughout its realization a number of people were involved and helped morally to complete the work.

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Curriculum vitae

Misghina Goitom Teklu was born in January 1979, in Debri Adi-hannes (Anseba), the red sea nation of Eritrea. In April 1996, he completed his high school studies at Asmara Comprehensive Secondary School, Asmara, Eritrea and joined the military training in the 5th round of the Eritrean national service. He passed the Eritrean national matriculation exam and attended the University of Asmara (UOA), College of Agriculture and Aquatic Sciences (CAAS) in September 1996. After completion of his studies in June 2001, he was drafted into the Eritrean national service in September 2001 as a teacher at Hamelmalo Agro-Technical School, Keren, Eritrea. Later, after he got his BSc degree in August 2002 - majoring with distinction in Animal Science - as a teacher at Warsay-Yikaalo Secondary School of Sawa, Gash Barka, Eritrea (2002-2003). From 2004-2005 he continued his national service as graduate assistant at the Eritrean Institute of Technology (EIT). From 2005-2008 he worked as graduate assistant in lecturing and research, at Hamelmalo Agricultural College (HAC) in Keren, Eritrea. In May 2008, he was granted an Erasmus Mundus Scholarship provided by the European Union (EU) and implemented by a consortium of European Universities, headed by the University of Ghent, Belgium. In September 2010, after finishing his Master thesis at Wageningen University and Research, The Netherlands, he obtained his European Master of Science in Nematology (EUMAINE) with distinction. His research topic was "Host status of fodder radish varieties (*Raphanus sativus* var. *Oleiformis*) against root-knot nematode, *Meloidogyne chitwoodi*. After his graduation, he received a scholarship as a guest Researcher at Wageningen University and Research as a nematologist at the department of Agrosystems Research. In September 2011, he started his Ph.D. programme at the same University under the supervision of Prof. Dr T.H. Been and Prof. Dr C.H. Schomaker and Promoter Prof. Dr Jaap Bakker.

His Ph.D. research is targeted on assessing the effect of crops resistant to *Meloidogyne chitwoodi* and, especially, on the development of a reliable method to test resistance of potato genotypes and green manure crops to *M. chitwoodi*. The research is carried out in close cooperation with Dutch potato breeding companies and funded by two public private collaboration (PPS) projects: MeloResist and MeloVir. The projects also included the development of new techniques, screening of populations and field validation of resistance tests. Besides his Ph.D., he supervised MSc students and cooperated in teaching the quantitative nematology course at Ghent University. He currently is a researcher at Agrosystems Research of Wageningen University & Research.

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- Teklu, M.G.,** Been, T.H. & Schomaker, C.H. (2011). Host status of fodder radish (*Raphanus sativus* var. *Oleiformis*) towards *Meloidogyne chitwoodi*. In: *Proceedings of the 63rd International Symposium on Crop protection*, Ghent, Belgium.
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- Teklu, M.G.,** Schomaker, C.H. & Been, T.H. (2016). Tuber yield, infestation and quality assessment of potato genotypes, partially resistant to *Meloidogyne chitwoodi*. In: *Proceedings of the 32nd Symposium European Society of Nematologist*, Braga, Portugal.
- Been, T.H., Schomaker, C.H. & **Teklu, M.G.** (2016). A routine test for Relative Susceptibility estimation of potato genotypes with resistance to *Meloidogyne chitwoodi*. In: *Proceedings of the 32nd Symposium of the European Society of Nematologist*, Braga, Portugal.

Laboratory manuals for resistance test

- Teklu, M.G.,** Beniers, J.E., Been, T.H. & Schomaker C.H. (2015). *Standard Operating Procedures (SOP) for the estimation of resistance of potato genotypes against Root-Knot Nematodes*. Wageningen University & Research, Agrosytems Research, 41 pp.
- Beniers, J.E., **Teklu, M.G.,** Been, T.H. & Schomaker C.H. (2015). *Standard Operating Procedures (SOP) for the estimation of resistance of potato genotypes against Potato Cyst Nematodes*. Wageningen University & Research, Agrosytems Research, 47 pp.

PE&RC Training and Education Statement

With the training and education activities listed below the Ph.D. candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Population dynamics of *Tylenchina* (plant root nematodes) and the consequences for host status screening and resistance testing; some major pitfalls discussed

Writing of project proposal (4.5 ECTS)

- Host status and resistance testing of agricultural crops against *Meloidogyne chitwoodi*

Post-graduate courses (3 ECTS)

- Introduction to R for statistical analysis; WUR/PE&RC
- Linear models; WUR/PE&RC
- Generalized linear models; WUR/PE&RC
- Meta-analysis; WUR/PE&RC
- Nema-Decide 1 and 2; Ghent University

Laboratory training and working visits (4.5 ECTS)

- Damage thresholds and population dynamics of *Pratylenchus penetrans* on carrot (*Daucus carota* L. cv. Nerac) at three different seed densities; Julius Kühn-Institut, Germany

Invited review of (unpublished) journal manuscript (3 ECTS)

- European Journal of Plant pathology: Resistance of *Coffea arabica* genotypes to *Meloidogyne* spp. under controlled and field conditions
- European Journal of Plant pathology: Future geo-climatic risks of the Potato Cyst Nematode in Scotland
- Plant Disease: Reproduction of *Pratylenchus brachyurus* on species and hybrids of eucalypts

Deficiency, refresh, brush-up courses (3 ECTS)

- Basic statistics; WUR/PE&RC
- Quantitative nematology; Ghent University

Competence strengthening / skills courses (3.3 ECTS)

- Project and time management; WUR/PE&RC
- Career assessment; WUR/PE&RC
- Techniques for writing and presenting a scientific paper; WUR/PE&RC
- WGS Ph.D. Workshop carousel; WUR/PE&RC

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC First year's weekend (2011)
- PE&RC Day (2013)
- PE&RC Final year's weekend (2014)
- PE&RC Day (2015)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- R-discussion group (2011-2017)
- Modelling and Statistical Network (MSN) discussion group (2012-2013)

International symposia, workshops and conferences (19.8 ECTS)

- 63rd International Symposium on Crop Protection; Ghent University, Belgium
- 64th International Symposium on Crop Protection; Ghent University, Belgium
- 65th International Symposium on Crop Protection; Ghent, University, Belgium
- 67th International Symposium on Crop Protection; Ghent University, Belgium
- 31st International Symposium of the European Society of Nematologist; Adana, Turkey
- 32nd International Symposium of the European Society of Nematologist; Braga, Portugal
- 52nd Annual meeting of Society of Nematologist; Knoxville, Tennessee, USA
- 54th Annual meeting of Society of Nematologist; Michigan State University, USA
- 6th International Congress of Nematology; Cape Town, South Africa

Lecturing / Supervision of practicals / tutorials (3.9)

- Techniques in nematology
- Quantitative nematology

Supervision of MSc students (7.5 ECTS)

- Damage thresholds and population dynamics of *Meloidogyne chitwoodi* on carrot (*Daucus carota* L. cv. Nerac) at different seed densities
- Determination of the effect of growing partially resistant potato cultivars on the population densities of *Globodera pallida*
- Efficiency of re-build Seinhorst elutriator in the extraction of *Pratylenchus penetrans* from soil
- Studying root and tuber resistance of potato genotypes against *Meloidogyne chitwoodi* in the presence of *Avena strigosa*

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