Life cycle and damage of the root-knot nematode *Meloidogyne minor* on potato, *Solanum tuberosum*

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Summary – *Meloidogyne minor* is a root-knot nematode reported in Belgium, Ireland, The Netherlands, Portugal, United Kingdom, Chile and the United States. It is found in sport fields and golf courses where it causes the yellow patch disease. However, *M. minor* has also been detected in potato fields in The Netherlands and the UK and may pose a threat for potato cultivation. Therefore, the life cycle and damage of *M. minor* on potato cv. Bintje were examined under controlled conditions. To assess its life cycle, young potato plants were inoculated with freshly hatched second-stage juveniles (J2). The developmental stages of *M. minor* were recorded at weekly intervals after inoculation until second generation J2 were detected. One week after inoculation, only vermiform juveniles were found in the roots. All juveniles were swollen after 3 weeks and the first adult females were observed. Egg masses were seen after 6 weeks together with second generation J2. The number of degree days for *M. minor* to complete its life cycle was calculated using a base temperature of 5°C (DD 5); between 606 and 727 DD 5 were needed to complete the life cycle. Damage development of *M. minor* on potato was examined in a pot experiment with different inoculation densities. Symptoms (galling on the tubers) were similar to those caused by *M. chitwoodi* and *M. fallax*. At initial population densities \( P_i \) of 10 J2 (100 cm\(^3\) soil\(^{-1}\)) and more, tubers showed galls. Severely damaged potato tubers were observed at \( P_i \geq 50 \) J2 (100 cm\(^3\) soil\(^{-1}\)) and a damage threshold of 41 J2 (100 cm\(^3\) soil\(^{-1}\)) was calculated. An *in vitro* test showed that five, commonly grown, potato cultivars were good hosts for *M. minor*. Based on our results, *M. minor* is able to develop on potato and cause severe damage at low initial population densities. Therefore, further spread of this nematode in agricultural fields should be avoided.

Keywords – damage threshold, degree days, development, host plant status, population density.

Potatoes are subjected to many pests, including nematodes. In addition to cyst nematodes (*Globodera* spp.), root-knot nematodes (*Meloidogyne* spp.) can cause severe damage to potato production. In warmer climates, *Meloidogyne enterolobii* (Rodriguez et al., 2003; Karssen et al., 2008) and *M. javanica* (Vovlas et al., 2005) may be a threat for potato culture; in cooler climates *M. hapla* (Santo & O’Bannon, 1981; Nyczepir et al., 1982), *M. chitwoodi* (Finley, 1981) and *M. fallax* (Wesemael et al., 2011) are causing major problems. *Meloidogyne chitwoodi* and *M. fallax* are listed as quarantine organisms in the EU (EC Directive 2000/29/EC) and EPPO region (A2 list) and, therefore, seed potato tubers should be nematode-free before they are allowed to enter EU traffic (Anon., 2000).

*Meloidogyne minor* is a recently described root-knot nematode species (Karssen et al., 2004). It is reported in Belgium, Ireland, The Netherlands, United Kingdom (Wesemael et al., 2011), Portugal, Chile (Karssen, pers. comm.) and United States (McClure et al., 2012). It was found in sport fields and golf courses where it causes yellow patch disease. However, *M. minor* was also detected in potato fields in The Netherlands and the UK and hence may pose a threat for potato cultivation. *Meloidogyne minor* is genetically closely related to *M. chitwoodi* and *M. fallax* (Holtermann et al., 2009) and can cause severe quality damage in potato with symptoms on tubers similar to those caused by *M. chitwoodi* and *M. fallax* (de Weerdt et al., 2011). It is likely that its life cycle will be similar to that of *M. chitwoodi* and *M. fallax* but

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field observations in The Netherlands indicated only one generation on potato (Thoden et al., 2012). By contrast, *M. chitwoodi* and *M. fallax* are known to have multiple generations on potato (Brommer & Molendijk, 2001; Griffin, 1985). The second generation of these species has been found responsible for the damage on potato tubers (Brommer & Molendijk, 2001). The damage threshold for *M. chitwoodi* on potato was estimated at 1 second-stage juvenile (J2) (250 cm$^{-3}$ soil$^{-1}$) (Santo et al., 1981) but depends on soil and environmental conditions, crop cultivar and cropping system (Greco & Di Vito, 2009). However, information on the damage threshold for *M. minor* is not available.

The objective of this work was to examine the life cycle duration of *M. minor* on potato, its damage threshold and the host plant status of economically important cultivars to evaluate its threat to potato production.

**Materials and methods**

**CULTURE OF MELOIDOGYNE MINOR**

Nematodes used for the experiments were obtained from stock cultures maintained on tomato plants (*Solanum lycopersicum* cv. Marmande) under controlled glasshouse conditions (20-28°C, 14 h light period). The population of *M. minor* originated from The Netherlands (provided by Dr G. Karssen, NVWA, Wageningen, The Netherlands). For the different experiments, freshly hatched (<24 h) J2 were used. Infected tomato plants were uprooted and the roots were carefully washed to remove the soil. The nematodes were extracted from the roots by cutting the roots into small pieces and placing them on a modified Baermann funnel (Hooper, 1986).

**HOST PLANT STATUS OF POTATO CULTIVARS**

The host plant status of five potato cultivars (cvs Asterix, Bintje, Nicola, Lady Rosetta and Première) for *M. chitwoodi*, *M. fallax* and *M. minor* was determined by evaluating egg mass formation. Seed potato tubers of the different cultivars were washed, disinfected for 5 min in a 5% sodium hypochlorite solution, thoroughly washed with water and left to sprout. After sprouting 60 tubers were planted in pots (9 cm diam.) filled with sterilised river sand and kept in a growth chamber where the temperature was recorded every hour using a data logger (Testo 175-T1; Testo) during the time of the experiment. Three weeks after planting, when roots had developed, each pot was inoculated with 500 freshly hatched J2 (<24 h) of *M. minor*. One week after inoculation, the plants were removed from the pots, their root systems were washed carefully and the plants were replanted in bigger pots (13 cm diam.) filled with sterilised soil. This was done to remove J2 that had not penetrated the roots thereby allowing detection of J2 of the second generation in the soil. On a weekly basis the developmental stage of *M. minor* was observed by staining the nematodes inside the roots with acid fuchsin (Byrd et al., 1983). Ten pots were taken randomly every week for nematode observation and the whole root system was monitored. After staining, the root fragments were kept in glycerol in a Petri dish and stored in a cold room at 4°C until observation of the developmental stages with the aid of a binocular microscope.

To check for the presence of second generation J2, 200 cm$^{-3}$ soil was taken from each of the ten pots examined, 5 and 6 weeks after inoculation. Soil samples were subjected to zonal centrifugation (Hendrickx, 1995) to extract nematodes based on their density. The experiment was repeated in time and lasted 5 (Experiment 1) and 6 weeks (Experiment 2). The degree days for *M. minor* to complete its life cycle was calculated using a base
temperature (minimum temperature for development) of 5°C. The basal temperature of this species has not been reported but we assumed it to be similar to that of *M. chitwoodi* (O’Bannon & Santo, 1984; Pinkerton et al., 1991).

**Damage threshold of *M. minor* on potato cv. Bintje**

To examine the potential threat of *M. minor* for potato culture, the damage threshold on cv. Bintje was determined. Cultivar Bintje was chosen as it is the most important cultivar in Belgian agriculture. Seed tubers were washed, disinfected with sodium hypochlorite as described above and left to sprout before planting in pots of 17 cm diam. filled with 2000 cm³ sterilised sandy soil. The pots were kept in a temperature and light controlled glasshouse (20-28°C, 14 h light). Three weeks after planting, the pots were inoculated with a series of initial population densities (Pᵢ) of freshly hatched J₂ of *M. minor* (0, 1, 2, 5, 10, 50, 125, 150 and 250 J₂ (100 cm³ soil⁻¹)). Each Pᵢ was replicated seven times. Plants were watered as required and fertilised with a slow release fertiliser (3 g per pot, NPK 16-10-13, Osmocote; Scotts). After 3 months, when the potatoes were maturing, plants were harvested for observation. The roots and tubers were removed from the soil, washed and weighed. The tubers were scored by visual inspection for the presence of galls and damage and the percentage tubers with galls and damage per plant was calculated. Potato tubers with more than 30% galling on their surface were considered severely damaged.

**Statistical analysis**

Egg mass formation of *M. chitwoodi*, *M. fallax* and *M. minor* on the different potato cultivars was subjected to a factorial ANOVA after log transformation of the data. Differences between species were analysed with a Fisher LSD test (P < 0.05). For the life cycle assay, the total number of *M. minor* (all life stages) observed inside the potato roots at the different times was analysed with ANOVA. For all analyses the Statistica 11 program was used. To determine the damage threshold the Seinhorst equation (Seinhorst, 1965) was calculated using the SeinFit program (Viaene et al., 1997).

**Results**

**Host plant status on potato cultivars**

Egg masses of *M. chitwoodi*, *M. fallax* and *M. minor* were found on the five tested potato cultivars 10 weeks after they had been inoculated (Fig. 1). The number per root system ranged from seven egg masses of *M. minor* on cv. Première to 139 egg masses of *M. fallax* on cv. Asterix. There was a significant difference in the number of egg masses between nematode species (F = 58.2, P < 0.01) and potato cultivars (F = 31.2, P < 0.01). The interaction between nematode species and potato cultivar was not significant (F = 1.6, P = 0.12). *Meloidogyne fallax* produced significantly more (P < 0.05) egg masses than *M. chitwoodi* and *M. minor* on all five cultivars. Egg mass formation of *M. fallax* was highest on cvs Asterix, Nicola, and Lady Rosetta. There was no difference in number of egg masses between *M. chitwoodi* and *M. minor* for each of the tested potato cultivars. For *M. chitwoodi*, fewer egg masses were found on cv. Bintje compared with cvs Asterix and Lady Rosetta. There was no difference between cvs Asterix, Nicola, Lady Rosetta and Bintje for *M. minor*. The lowest number of egg masses for all three species was found on cv. Première.

**Life cycle of *Meloidogyne minor* on cv. Bintje**

During both experiments, separated in time, the temperature was stable at 22.3 ± 0.65°C. In the first experiment

![Fig. 1. Mean number of egg masses (± SE) of *Meloidogyne minor*, *M. chitwoodi* and *M. fallax* per root system of five potato cultivars (cvs Asterix, Nicola, Lady Rosetta, Bintje and Première). Different letters indicate significant differences (LSD-test, P < 0.05).](image-url)
vermiform juveniles were found in the roots 1 week after inoculation (Fig. 2). After 2 weeks, swollen juveniles were detected but vermiform juveniles were still present. All juveniles were swollen after 3 weeks and the first adult females were observed. In the 4th and 5th week after inoculation a small proportion of swollen juveniles was still present, together with females. No J2 were found in the soil 5 weeks after inoculation. The total number of nematodes found inside the roots after 1 week was higher than the number found after 2, 3, 4 and 5 weeks ($F = 5.15$, $P < 0.01$).

In the second experiment the developmental stages of *M. minor* found in the roots after 1 and 2 weeks were similar to those in Experiment 1 (Fig. 2). Three weeks after inoculation the number of females was higher than in Experiment 1. The 5th and 6th week after inoculation, a small proportion of swollen juveniles were still present. After 6 weeks females with egg masses were observed and new vermiform juveniles were found in the roots. Second generation J2 (ranging from 1 to 5 J2 (100 cm$^3$ soil)$^{-1}$) were found in the soil of four replicates 6 weeks after inoculation. The total number of nematodes in the roots did not change during the experiment ($F = 1.00$, $P = 0.424$) as seen in Experiment 1. Based on the observation of J2 of the second generation after 6 weeks, the number of degree days (base temperature = 5°C) for *M. minor* to complete its life cycle was between 606 and 727 DD$_5$.

**DAMAGE THRESHOLD OF *M. minor* ON POTATO CV. BINTJE**

At low $P_i$ (1, 2 and 5 J2 (100 cm$^3$ soil)$^{-1}$) no galls were observed on the tubers of cv. Bintje 3 months after inoculation with *M. minor* (Fig. 3). Tubers with galls were detected at a $P_i$ of 10 J2 (100 cm$^3$ soil)$^{-1}$ and their percentage increased with increasing $P_i$. Severely damaged tubers (>30% galling) were observed at $P_i \geq 50$ J2 (100 cm$^3$ soil)$^{-1}$ and their percentage increased with increasing $P_i$. At $P_i = 250$ J2 (100 cm$^3$ soil)$^{-1}$, 75%
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of the tubers showed severe damage. Damaged tubers showed severe galling (Fig. 4) with many females below the skin of the tubers.

The damage threshold \((T)\) for quality damage on tubers was 41 J2 (100 cm\(^3\) soil\(^{-1}\)), based on the Seinhorst model \((R^2 = 0.58)\) (Fig. 5). Parameters of the model (Table 1) indicate that the minimum yield was 25% of the maximum yield.

**Discussion**

*Meloidogyne minor* has been found in potato fields in Northern Ireland (Fleming, pers. comm.) and The Netherlands (Karssen *et al.*, 2004). At the location from which *M. minor* was isolated in 2000 in The Netherlands, the potato tubers (unknown cultivar) were seemingly not infected at the time of isolation and did not show any symptoms. In a field experiment in 2009, big pear-shaped galls were observed on the roots and white females were detected below the skin of tubers of cvs Asterix and Markies but no symptoms were noticed on the outside of the tubers (Thoden *et al.*, 2012). In our experiments we observed heavy infection with numerous skin blemishes and white females with egg masses below the skin of cv. Bintje. These symptoms were also reported by Karssen *et al.* (2004) and Thoden *et al.* (2012) from pot tests with *M.*
Table 1. Parameter estimates of the Seinhorst model for damage on tubers of potato cv. Bintje grown in 2000 cm³ pots caused by _Meloidogyne minor_ at 11 initial population densities (P₁) from 0-250 second-stage juveniles (J2) (100 cm³ soil⁻¹).

<table>
<thead>
<tr>
<th>Parameter a</th>
<th>y_m · m</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>T</td>
<td>z</td>
</tr>
<tr>
<td>0.25</td>
<td>41</td>
<td>0.99</td>
</tr>
</tbody>
</table>

a The Seinhorst model is of the form: \( Y = y_m \) for \( P_1 \leq T \) and \( Y = (y_m · m) + y_m · (1 - m) · z^P_1 - T \) for \( P_1 > T \); \( y_m \) = maximum yield without nematode damage, \( m \) = a constant so that \( y_m · m \) equals the minimum yield, \( z \) = parameter determining the slope of the curve, \( T \) = damage threshold (J2 (100 cm³ soil⁻¹)).

_minor_ and are similar to those caused by _M. chitwoodi_ and _M. fallax_ on potato; this was also reported by de Weerdt et al. (2011). However, the damage threshold that we determined for _M. minor_ on potato is markedly higher compared with _M. chitwoodi_ and _M. fallax_. Based on the Seinhorst model we found a threshold of \( P_1 = 41 \) J2 (100 cm³ soil⁻¹). Santo et al. (1981) reported a damage threshold of less than \( 1 \) J2 (250 cm³ soil⁻¹) for _M. chitwoodi_ on potato cv. Russet Burbank and in The Netherlands a threshold of \( 10 \) J2 (100 cm³ soil⁻¹) is applied for this species (Norsheid et al., 2011). For _M. chitwoodi_ it was shown in a field experiment that degree-day accumulation during the growing season was more important than initial population densities for damage development on potato tubers cv. Russet Burbank (Griffin, 1985). The damage is caused by the second- and third-generations, which are formed when tubers are present. Possibly, the same reasons apply for damage of _M. minor_ on tubers. The damage was determined after 3 months in a pot test giving ample time for at least a second generation to develop.

When calculating the thermal time (degree days), knowledge of the base temperature (\( T_b \)) is important (Trudgill et al., 2005). Pinkerton et al. (1991) estimated from field studies on potato a \( T_b \) of 5°C for _M. chitwoodi_. The \( T_b \) for _M. minor_ has not been reported but we assumed it to be similar to that of _M. chitwoodi_. Similarities in reproduction biology on potato between _M. minor_ and _M. chitwoodi_ have been reported by Thoden et al. (2012). J2 of _M. minor_ were able to survive in water at 4°C for more than 12 weeks (Wesemael et al., 2012). Morris et al. (2011) showed limited activity (movement) of J2 of _M. minor_ at 4°C and 100% activity at 10°C. This supports our choice for 5°C as \( T_b \) for development. However, it is unknown if J2 of _M. minor_ can penetrate and develop within root tissue at this temperature. Hence, more research on this aspect is required. Morris et al. (2011) demonstrated that hatching of J2 was less than 1% when egg masses were incubated for 63 days at temperatures below 10°C. The optimum temperature for hatching was determined at 20-25°C and the optimum temperature for activity was 15-25°C (Morris et al., 2011). Our experiment to determine the life cycle duration of _M. minor_ was performed at 22.3°C which is in the optimum temperature range for activity. At 22.3°C it took 35-42 days for _M. minor_ to complete its life cycle. This is comparable with _M. arenaria_, _M. hapla_, _M. incognita_ and _M. javanica_, whose life cycles took 36, 43, 37 and 43 days, respectively, at 21°C (Ploeg & Maris, 1999). Observations at shorter time intervals are required to compare precisely among species. Based on the nematode development at set temperature, we calculated that _M. minor_ requires 606-727 DD₅ to complete its life cycle on potato. This is similar to that found for _M. chitwoodi_, which required 600-800 DD₅ (Pinkerton et al., 1991). As for _M. chitwoodi_ and _M. fallax_, it seems that _M. minor_ is able to produce several generations during one growing season. This corroborates findings by Turner & Fleming (2005). However, based on reproduction under field conditions on potatoes, Thoden et al. (2012) assumed only one generation per year was produced. Their experimental fields were located on the island Texel in the northwest of The Netherlands. Based on the mean daily air temperatures of the weather station at Vlieeland (data KNMI, The Netherlands), 20 km from their experimental fields, approximately 1890 DD₅ were reached during the potato field period, which should have allowed a second generation. Therefore, the females they observed in the tubers were probably second generation females. In east Ireland, egg masses of _M. minor_ were detected during the whole year on creeping bentgrass (Morris et al., 2011), but the mean number of eggs per egg mass peaked in May and September. The total degree days (base 5°C) per year in east Ireland is 1631, based on monthly average soil temperatures at 10 cm depth from the period 1981-2010 (data from Casement weather station, MET éireann). In the period 1 June–30 August a total of 955 DD₅ is reached and from 1 September until 31 May 677 DD₅ are accumulated. If we assume that the peaks in the number of eggs indicate the completion of a generation then the observations by Morris et al. (2011) confirm our estimation for the duration of the life cycle of _M. minor_.

The results on the host plant status showed that the egg mass formation of _M. minor_ on five important commercial potato cultivars was similar to the egg mass formation
of \textit{M. chitwoodi} but less than that of \textit{M. fallax}. We did not assess the number of eggs per egg mass. The three \textit{Meloidogyne} species had fewer egg masses on cv. Première when compared with the other cultivars. This could have been influenced by the fact that the seed tuber of cv. Première started deteriorating during the time of the experiment in the closed containers. Cultivar Première is an early potato with a shorter field period than the other cultivars (NIVAP, 2003). Given the generally high numbers of egg masses we can consider all screened cultivars to be good hosts for \textit{M. chitwoodi}, \textit{M. fallax} and \textit{M. minor}.

Our results on the biology show that \textit{M. chitwoodi}, \textit{M. fallax} and \textit{M. minor} may be closely related. Molecularly \textit{M. minor} is closely related to \textit{M. chitwoodi} and \textit{M. fallax} (Karssen et al., 2004; Holtermann et al., 2009; McClure et al., 2012). It is clear from our results that the production of commercially important potato cultivars can be threatened by \textit{M. minor} as it affects their quality and yield and is able to complete its life cycle within the average crop cycle. Therefore, further spread of this nematode in agricultural fields should be avoided.

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