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A field study on the host status of different crops for *Meloidogyne minor* and its damage potential on potatoes

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Summary – For several years, a new species of root-knot nematode, *Meloidogyne minor*, has been reported from parts of The Netherlands, Belgium, UK and Ireland. So far, this species causes most problems on golf courses but has also been reported from a potato field in Zeijerveld (The Netherlands) where it caused strong growth reduction on potato plants, but no damage to potato tubers. As The Netherlands is a potato-producing country, field experiments were set up to evaluate the potential risks this species poses. We tested the host status of some common crops for *M. minor* under field conditions and, more importantly, also tested its potential to harm potato production in terms of quantity as well as quality. In a 2-year field experiment (2007-2009), the host status of potato (cv. Bartina), rye, annual ryegrass, sugar beet, and maize was tested in the first growing season. Afterwards, these plots were used to evaluate the damage potential of *M. minor* on two commonly cultivated potato cultivars (cvs Astérix and Markies). In general, only potato seemed to be a good host for this nematode species with a *Pf/Pi*-ratio about 1.5. Reproduction was observed mostly on roots but also on tubers, which increases the spread of *M. minor* by seed potatoes. However, there was no reduction in potato production, neither in yield nor in tuber quality. No significant reproduction could be observed on the other plants (*Pf/Pi* values close to zero). From these results one might conclude that this nematode will not become a major threat to European agriculture. However, care has to be taken as within additional glasshouse experiments potato tubers were susceptible for damage caused by *M. minor*. Thus, further studies on the general biology and ecology of *M. minor* are needed to make a better risk assessment on this new nematode pest.

Keywords – annual ryegrass, host status, maize, nematode damage, root-knot nematode, rye, sugar beet.

Root-knot nematodes, *Meloidogyne* spp., cause more economic damage than any other single group of plant-parasitic nematodes. In The Netherlands and other parts of northern Europe, so far the northern root-knot nematode *Meloidogyne hapla*, the barley root-knot nematode, *M. naasi*, the Colombia root-knot nematode, *M. chitwoodi*, and its close relative the false Colombia root-knot nematode, *M. fallax*, were the most recognised species of root-knot nematodes due to the great economic damage they cause on numerous field crops (Perry *et al.*, 2009; Wesemael *et al.*, 2011). In 2000, however, a new species of root-knot nematode, *M. minor*, was found in a potato field in Zeijerveld, The Netherlands (Karssen *et al.*, 2004). At this time, infected potato plants clearly lacked growth, while no signs of tuber infection were present. Correspondingly, juveniles of *M. minor* could only be isolated from the roots (Karssen *et al.*, 2004). Since this first observation, *M. minor* has also been detected on several golf courses and sports grounds throughout the UK, Belgium and The Netherlands, which often could be related to the so-called yellow patch disease on creeping bent grass (*Agrostis stolonifera*) (Turner & Fleming, 2005; Viaene *et al.*, 2007; Vandenbossche *et al.*, 2011). Furthermore, considerable effort has been put into a pest risk assessment (PRA, 2006) and the development of molecular tools for the identification of *M. minor*, which will facilitate detection and determination (de Weerdt *et al.*, 2011).

Within glasshouse studies *M. minor* has been reported to reproduce on many plants (Karssen *et al.*, 2004), including mono- as well as dicotyledons such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), carrot (*Daucus carota*), phacelia (*Phacelia tanacetifolia*), alfalfa (*Medicago sativa*), annual and perennial ryegrass (*Lolium multiflorum*, *L. perenne*), oat (*Avena sativa*), lettuce (*Lactuca sativa*) and vetch (*Vicia sativa*), but failed to reproduce on marigold (*Tagetes patula*) and maize (*Zea mays*). Furthermore, reproduction on potato has been observed on roots as well as potato tubers

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(Karssen *et al.*, 2004). However, field experiments on both the damage potential of *M. minor* to potato production and its potential host plants are missing (probably due to difficulties in finding suitable experimental fields, as *M. minor* often appears within mixed populations of *M. minor* and *M. naasi*). Therefore, it is still difficult to evaluate if *M. minor* might become a threat to Dutch agriculture. Thus, in cooperation with the Dutch Plant Protection Service, a field study was set up to evaluate: *i*) the host range of several common crops for *M. minor*; and *ii*) the damage potential of *M. minor* to potato.

Materials and methods

Site description

The experimental field was located close to Oosterend, on the island of Texel, in the northwest of The Netherlands. The soil was a sandy soil (pH-KCl = 4.65, OM = 2.15%) with a mixed population of *M. minor* and *M. naasi* with some other plant-parasitic nematodes. The field site had been in grassland (a mixture of several grass species) in 2007 and was cropped with potato in 2006, when the farmer had observed symptoms of root-knot nematode infection on the tubers. The site (36 × 78 m) was split into 48 plots (6 × 6 m) arranged in four blocks, which were separated by individual 'buffer' strips (6 m wide) (Fig. 1). Each block was further separated in two sub-blocks that were also separated by a buffer strip (3 m wide).

Experimental design for *M. minor* host plant study

The host plant study was established in April 2008 when the whole experimental site was fertilised with 400 kg ha^{-1} ammonium nitrate. Three weeks later, the existing grass cover was sprayed with the herbicide glyphosate and ploughed and harrowed 2 weeks later. Subsequently, the various plots were cropped with either rye (*Secale cereale* cv. Sorum; 130 kg ha^{-1}), sugar beet (*Beta vulgaris* cv. Shakira; 110 seeds ha^{-1}), maize (*Z. mays* cv. Expert; 115 seeds ha^{-1}), annual ryegrass (*L. multiflorum* cv. Bartali; 30 kg ha^{-1}) or potato (*S. tuberosum* cv. Bartina; 0.75 × 0.3 m), respectively (Fig. 1). Each treatment was replicated eight times as was a fallow treatment, which was included as a control treatment. During the growing period, emerging weeds were either removed by hand or by herbicide. Finally, plants were harvested mid-October without determination of crop yields.

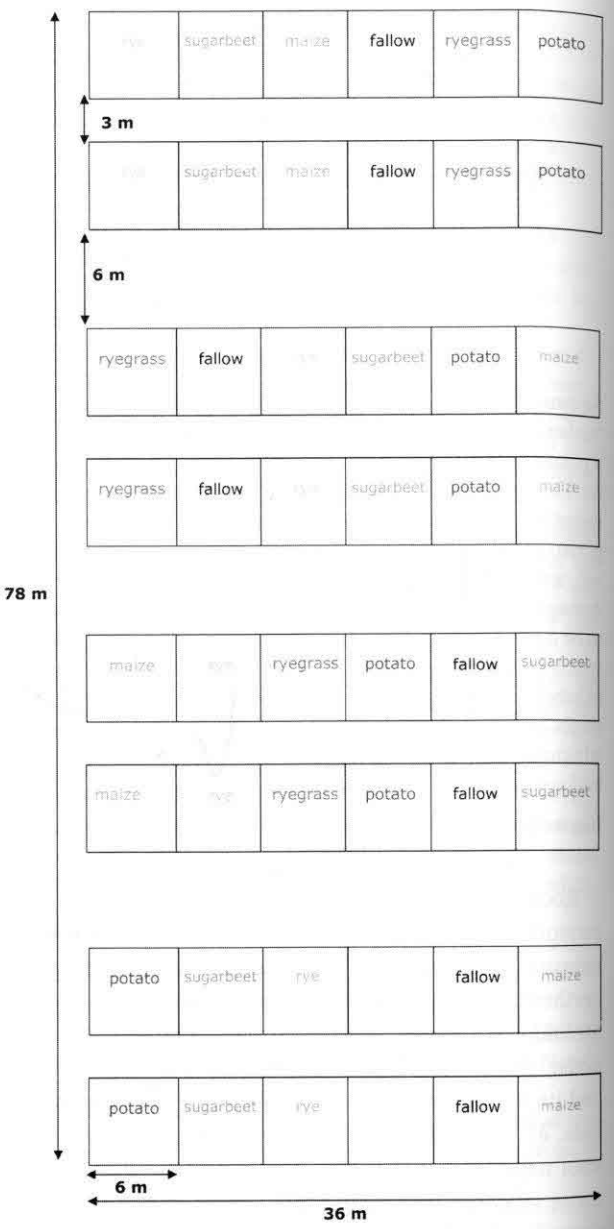

Fig. 1. *Set up of the experimental field including the tested host plants. In the damage study on potato one row of each block was used to grow cv. Markies or Astérix, respectively. This figure is published in colour in the online edition of this journal, which can be accessed* via *http://www.bril.nl/nemy*

Experimental design for damage study of *M. minor* on potato

After the host study in 2008, the same site with all 48 plots was used in 2009 to evaluate the damage potential of *M. minor* to potato. Remaining plant material from

the host plant study was removed, and the field was treated with glyphosate mid-March and tilled as well as disc-harrowed the following week. Four weeks later, two commonly used potato cultivars, Astérix and Markies, were planted mechanically at a spacing of 0.75 × 0.30 m (four rows per individual plot). Each of the four main blocks was split in two sections, where one half was planted with cv. Markies and the other half was planted with cv. Astérix; cv. Astérix was chosen because of its strong susceptibility to *M. chitwoodi* and cv. Markies was chosen because of its somewhat longer growing period, which probably supports stronger nematode reproduction. To enhance this difference, plots containing cv. Astérix were sprayed with the herbicide dibromide at the end of September and potatoes were harvested mechanically 3 weeks later. Cultivar Markies was harvested at the beginning of November when plants started to die off naturally. In both cases an area of 9 m^2 was harvested per plot. Tubers were first classified within different sizes to determine marketable yield and then evaluated for external signs of nematode infection (deformations) as well as for visible damage inside the tubers (presence of females). Thirty tubers were selected at random per plot and scored according to a tuber-damage-index (TDI). This index is calculated by two parameters: *i*) the external deformations of the tuber; and *ii*) the blemishes – in general, adult females – under the peeled skin. Depending on both parameters, tubers were classified from 0 to 4 (representing 0, 10, 33, 66 or 100% tuber damage) and the TDI per plot was calculated as $(\sum n_i * K_i)/N$, where n_i is the number of tubers within class i, K_i = 0, 10, 33, 66, 100 for $i = 0$ to 4 and N is the total number of tubers evaluated (per plot). Thus, the index ranges between 0 and 100. Additionally, to confirm that infection was caused by *M. minor*, several tubers from each cultivar were peeled and nematodes were extracted from several grams of peel within a mist chamber (over 4 weeks, peel placed on sieves).

Sampling of nematodes

Plots were sampled at the beginning of the host plant study in May 2008, after the corresponding harvest in November 2008, again mid-April 2009, and in May 2010 after the damage trial on potato. From our results in 2008-2009 we could see that there was almost no difference between the population density of root-knot nematodes measured in autumn 2008 or spring 2009 (see Discussion). Thus, we decided to measure the final population densities for the damage study on potato in spring (2010).

At each sampling date, 35 soil cores were taken in a regular pattern from the centre (1.5 × 2.67 m) of each plot (auger = 13 mm diam., 25 cm depth). Samples were stored in plastic bags at 4°C until a subsample of 100 ml soil was used for nematode extraction. Subsamples were first sieved (mesh size 180 μm) with water. Nematodes from the suspensions were then extracted using an Oostenbrink elutriator (Verschoor & de Goede, 2000 and references therein). In addition, the remaining organic matter fraction on the sieves (mainly roots) was incubated for 4 weeks at 20°C to allow hatch and emergence of motile endoparasitic stages from the roots. Nematode numbers per sample (Oostenbrink + incubation) were determined by counting two 10 ml aliquots from the obtained nematode suspensions (which were 100 ml each). Within the aliquots all plant-parasitic nematodes were counted and identified to genus level (at a magnification of 40×; Bongers, 1994). Then between 20-25 root-knot nematodes were handpicked, transferred to a glass slide and identified to species level (at 400-1000×). In the case of our *Pi*-sampling in April 2008 this was only done within ten samples (from 48 plots). The corresponding ratio of *M. minor* to *M. naasi* in those samples was then also used to calculate the *Pi*-values of *M. minor* and *M. naasi* for the remaining plots (homogeneous grass cover in 2007). For the final populations (*Pf*), however, the ratio of these two species was separately determined for each host plant, from at least half of the eight repetitions per plant. To support our morphological data some samples were also analysed by molecular techniques (PCR-DGGE) (de Weerdt *et al.*, 2011).

In addition to soil samples, plant tissue was also evaluated for signs of root-knot nematode infection (visually) and some (5-10 g) root and/or tuber material of maize, potato as well as sugar beet were placed on sieves in a mist chamber to extract nematodes.

Statistical analysis

A factorial design with four completely randomised blocks was applied, and all data were statistically analysed with GenStat (2009). Treatment means of the initial and final population densities (as well as *Pf*/*Pi*-ratios) were calculated and separated by pairwise *t*-test. Furthermore, a non-linear regression analysis (Model $Pf = 1 - e^{-(a/M)Pi}$; Poisson distributed) was performed to evaluate the relation between the initial (*Pi*) and final population densities (*Pf*). Similarly, a regression analysis using

the Seinhorst model ($Yi = Y\max\{m + (1 - m) * 0.95^{(Pi-T)/T}\}$) was used to estimate the relation between *Pi*-levels and the damage (yield) on potatoes (for detailed descriptions see Greco & di Vito, 2009).

Results

Host plant study in 2008

Figure 2 shows the relations between initial and final population densities of *M. minor* per plant. Based on our *Pf*-sampling in May 2009, only potato (cv. Bartina) supported considerable (*Pf*/*Pi*-value = 1.4) reproduction of *M. minor* during the host plant study in 2008. No visual signs of infected roots could be observed, nor did we extract any juveniles of *M. minor* from potato tubers collected at harvest. No substantial reproduction of *M. minor* was present on the other host plants (*Pf*/*Pi*-values < 0.1) of which only annual ryegrass had a statistically significant ($P < 0.05$) higher population density of *M. minor* than the fallow (Fig. 2). In addition, no nematodes were found in the roots of sugar beet or maize.

Reproduction of *M. naasi* (data not shown) was present on rye and annual ryegrass (*Pf*/*Pi* = 1.5 and 0.9). No considerable reproduction of *M. naasi* was present on the other crops which statistically could not be separated from the fallow treatment ($P < 0.05$).

Host status and susceptibility of two common potato cultivars

During a field observation of the damage trial in July 2009, big pear-shaped galls (0-2 cm) could only be detected on roots of the cv. Astérix (Fig. 3A, B). Most galls were located at the beginning of lateral roots leading to a thickened root base. Sometimes lateral roots continued to grow, which led to a 'tail' on top of the galls. In the laboratory we crushed some of those root galls between two glass slides and found dozens of nematode eggs (Fig. 3C) and also adult females (Fig. 3D). Additionally, some galls of *M. minor* were placed in a mist chamber, from which approximately 4000 second-stage juveniles (J2) (g gall material)$^{-1}$ were counted after 2 weeks extraction.

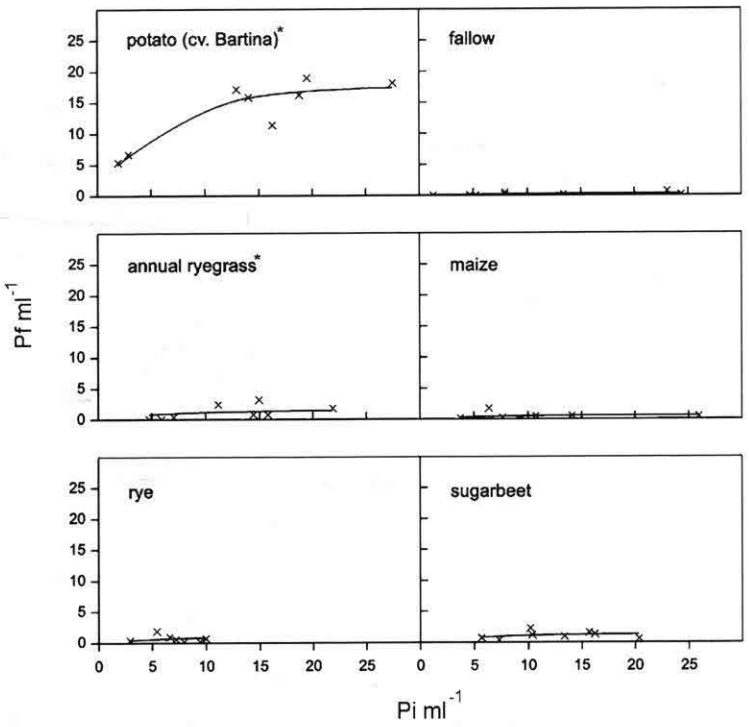

Fig. 2. *Relation between initial* (Pi *(ml soil)*$^{-1}$*) and final population densities of* Meloidogyne minor (Pf *(ml soil)*$^{-1}$*) for the host plant study in 2008* (Pf *is based on samples taken in spring 2009); *significantly different to fallow* ($P < 0.05$).

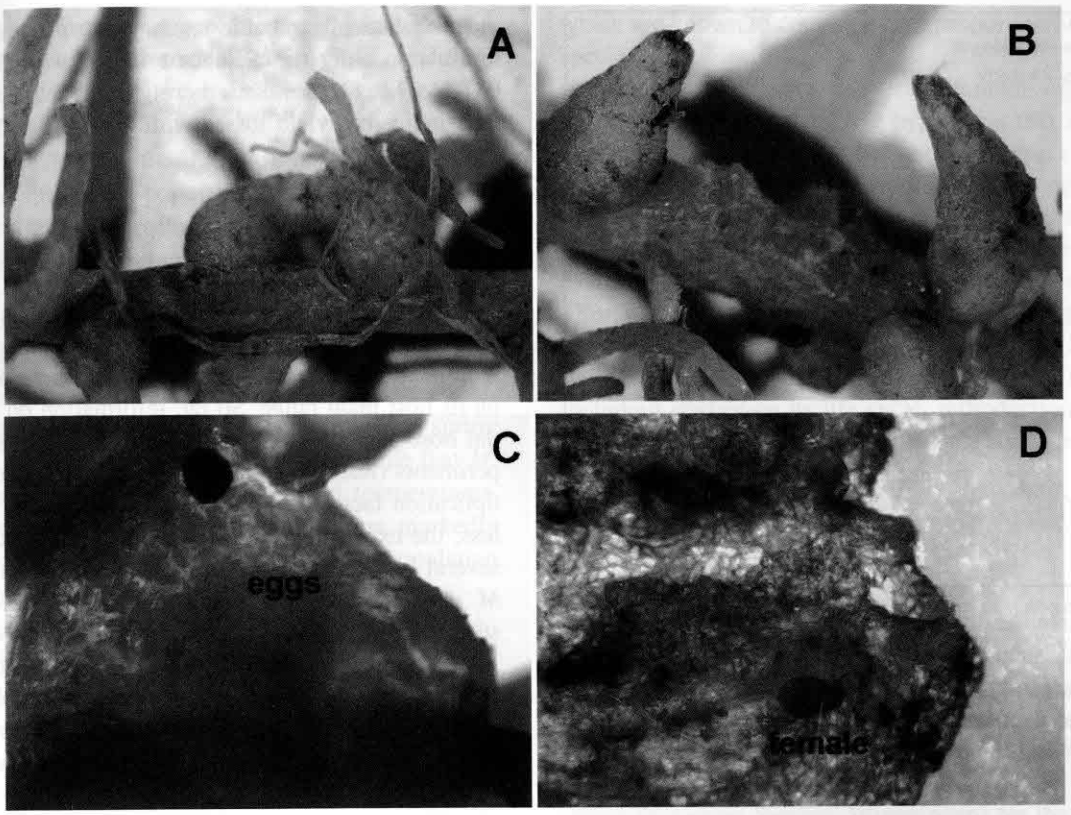

Fig. 3. *Symptoms caused by* Meloidogyne minor *on field samples of potato (cv. Astérix) taken in spring 2009. A, B: Root-galls on potato; C, D: Eggs and adult female within root gall. This figure is published in colour in the online edition of this journal, which can be accessed* via *http://www.brill.nl/nemy*

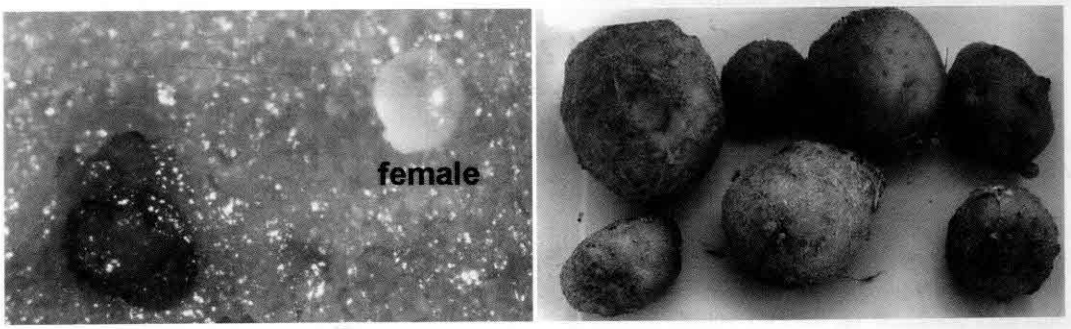

Fig. 4. *A female of* Meloidogyne minor *and damage on potato cv. Astérix in 2009 (glasshouse study). This figure is published in colour in the online edition of this journal, which can be accessed* via *http://www.brill.nl/nemy*

Apart from root symptoms on cv. Astérix, tubers from 12 out of 48 plots also showed slight symptoms of *M. minor* infestation at harvest (tuber index 11-55). These were visible as white dots (young or adult females) under the potato skin of both cultivars (Fig. 4); no signs of infection were present on the outside of tubers. Within the corresponding tuber material J2 of *M. minor* could only be extracted from the cv. Markies. Similarly to tuber infection, soil population densities showed reproduction of *M. minor* on both potato cultivars (Fig. 5); cv. Markies was a slightly better host than cv. Astérix.

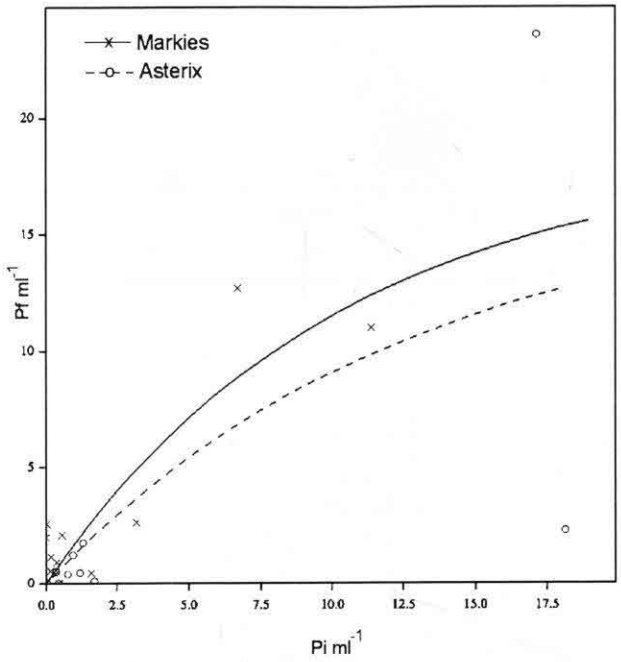

Fig. 5. *Relation between initial* (Pi *(ml soil^{-1})) and final populations* (Pf *(ml soil^{-1})) of* Meloidogyne minor *on two potato cultivars in 2009* (Pf *based on sampling in spring 2010).*

No statistically reliable results were produced by the regression using the Seinhorst damage model (R^2 = 0.097) (Fig. 6). However, there is a tendency within the data indicating yield losses with increasing *Pi*-levels of *M. minor*.

Discussion

This study aimed to obtain more insight into the potential risk *M. minor* might cause to potato production within The Netherlands and to obtain more knowledge on its host plant range. So far, information on the potential host plants for *M. minor* came from glasshouse experiments (Karssen *et al.*, 2004) where the average multiplication factor was low, and often below 1. Nevertheless, the experiments showed nematode reproduction on several hosts and therefore indicated a potential risk of *M. minor* to agriculture. By contrast, in the present field study only potato supported a significant reproduction of *M. minor* in the sense that the final population densities exceeded the initial populations observed. Thus, we consider potato to be a good host plant for *M. minor*. The measured reproduction supports the assumption that *M.*

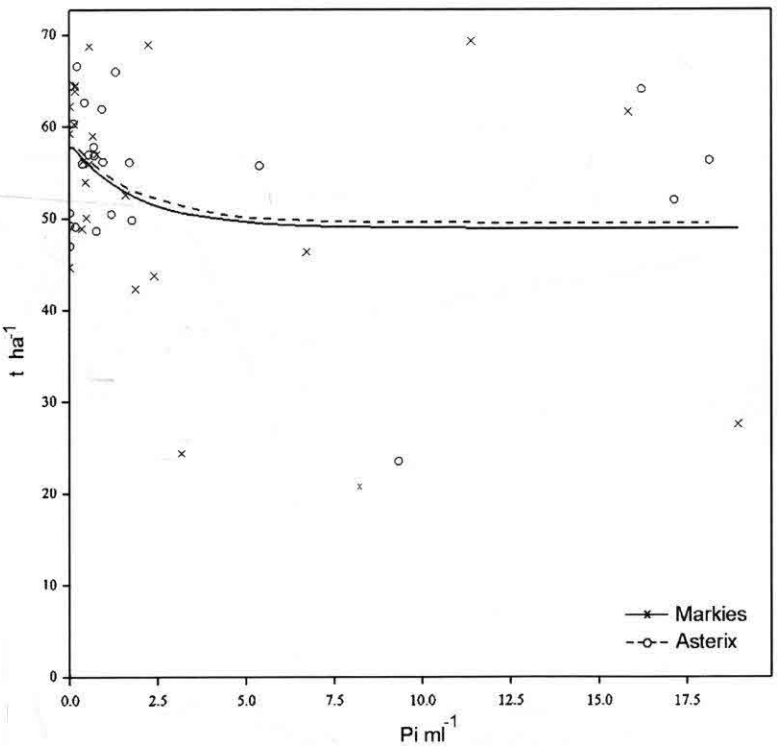

Fig. 6. *Effect of initial population* (Pi *(ml soil^{-1})) of* Meloidogyne minor *on the marketable fresh yield of potato* (R^2 = *0.097).*

minor only produces one generation per year. This resembles *M. naasi* which under Belgian field conditions also produces only a single generation per year (Gooris & D'Herde, 1977).

Another analogy with *M. naasi* is the stability of autumn and spring soil population densities. Eggs of *M. naasi* exhibit temperature dormancy, which is necessary to stimulate hatching in spring (Franklin *et al.*, 1971). Thus, population densities of *M. naasi* were almost the same for samples taken in autumn or spring if the former had been chilled before extraction (incubation; Franklin *et al.*, 1971). Similarly, we found nearly the same numbers of *M. minor* in samples taken in autumn or the following spring if samples were stored at 4°C. Thus, we assume that *M. minor* overwinters unharmed by the cold temperatures, probably within its egg masses. This differs markedly from *M. chitwoodi*, which shows a drastic population decrease during Dutch winters.

Interestingly, reproduction of *M. minor* could be observed on both roots and potato tubers where the amount of infected tubers was low under field conditions. By contrast, in their glasshouse study, Karssen *et al.* (2004) reported heavily infected potato tubers and we also found strong tuber infection on different potato cultivars including cv. Astérix in a recent glasshouse study (see Fig. 5; data not shown). These differences in tuber infection are probably influenced by factors such as temperature and moisture (Santo & O'Bannon, 1981). However, even low infection of tubers drastically increases the risk that *M. minor* might be spread by seed potatoes.

Reproduction in tubers as well as roots has also been described for *M. chitwoodi*. This similarity in biology might be linked to molecular data from van Megen *et al.* (2009) and Holtermann *et al.* (2009), which show a strong genetic connection between these two species.

In addition to potato, only annual ryegrass (*L. multiflorum*) supported population densities of *M. minor* that were significantly higher than the population densities in the fallow treatment. However, densities were still very low ($Pf/Pi < 0.1$). This seems curious as *M. minor* has been reported to cause yellow patch disease on creeping bentgrass (*Agrostis stolonifera*) and, consequently, we also expected reproduction on other members of the Poaceae. On the other hand, *M. minor* was only identified on turf grass that was made up of *Poa*, *Agrostis* and *Festuca* species and not on turf grass that mainly consisted of *Lolium* species (Vandenbossche *et al.*, 2011). Similarly, natural populations of *M. minor* have been reported from coastal dunes which are known to contain a high proportion of *Agrostis* species.

Given the small host range seen within this experiment, the low reproduction and the hypothesis that *M. minor* naturally only occurs in dunes, we do not expect *M. minor* to become a major threat to north European agriculture, unlike *M. chitwoodi* or *M. hapla*. However, the threat this nematode will pose under other conditions, such as higher temperatures, or to other crops is unknown. Thus, additional research is necessary to shed light on the risks of spreading *M. minor* by seed potatoes. Similarly, we have to learn more about the biology of *M. minor* including studies on its life cycle, reproduction (fertility and fecundity) and, of course, its ability to infest and reproduce on other important crops.

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