Pratylenchus penetrans, a potential risk in glasshouse-grown lettuce: population dynamics and damage threshold

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Summary – The root-lesion nematode, *Pratylenchus penetrans*, causes growth reduction in glasshouse-grown lettuce and is mainly controlled by chemical soil disinfestation. Integrated management strategies require more knowledge about the population dynamics and damage threshold densities. We monitored the population during 2.5 years in a commercial glasshouse by sampling soil in the same four 1 m² spots at 0-30 cm and 30-60 cm depth. The grower grew lettuce in rotation with leek, applied 1,3-dichloropropene in summer and left the field fallow during winter. Growing leek reduced the nematode population slightly but chemical soil disinfestation lowered the numbers drastically, although 41% of the nematodes in the deeper layer survived. Black fallow resulted in a slight increase of the population, probably due to hatching. Two pot experiments with ten densities of *P. penetrans* were conducted to estimate the damage threshold for a summer and autumn cultivar ('Cosmopolia' and 'Brighton', respectively). The thresholds for lettuce weight were 669 and 3834 *P. penetrans* (100 ml soil)⁻¹ in summer and autumn, respectively, but with considerable variability in estimated parameters. The thresholds for root damage were much lower: 204 and 48 *P. penetrans* (100 ml soil)⁻¹. Nematode numbers did not increase on lettuce in the pot tests (maximum multiplication rate was 0.40) but increased slightly in the commercial setting. These results show that populations of *P. penetrans* build up slowly when butterhead lettuce is rotated with leek and fallow, but chemical soil disinfestation is required to avoid numbers resulting in root damage.

Keywords – *Allium porrum* (leek), field monitoring, *Lactuca sativa*, population density, root-lesion nematode, Seinhorst equation, tolerance limit, yield loss.

In Belgium, butterhead lettuce (*Lactuca sativa*) is mainly produced in soil in glasshouses as a monoculture with up to five harvests per year. In some cases, however, the production of lettuce is rotated with other crops, such as leek or celery. Occasionally, nematodes are associated with severe growth reduction of glasshouse-grown lettuce. In a Belgian survey, soil samples from 38 glasshouses were analysed in order to have more insight into the

presence of plant-parasitic nematodes. The study revealed that the root-lesion nematode, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941, was present in 18% of the glasshouses (unpubl. data, 2014). *Pratylenchus penetrans* is spread worldwide and has a broad host range of nearly 400 plant species. This migratory endoparasitic nematode causes severe damage to economically important crops such as maize, potato

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and cereals, as well as several vegetables (Castillo & Vovlas, 2007). Damage on lettuce caused by *P. penetrans* was reported by Gracia *et al.* (1991), Kilpatrick *et al.* (1963), Moretti *et al.* (1981) and Olthof & Potter (1973). Based on scientific and economic importance, root-lesion nematodes (*Pratylenchus* spp.) are ranked third after root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.) (Jones *et al.*, 2013).

Currently, growers mainly control nematodes in lettuce by chemical soil disinfestation, which is also applied to reduce several other soil-borne pathogens. Stricter phytosanitary regulations in Belgium, driven by increasing environmental concerns, demand a reduction in the use of chemicals for soil disinfestation. To set up integrated pest management strategies, knowledge about the population dynamics and the damage threshold of *P. penetrans* for lettuce is required. The damage threshold density of a nematode species for a certain crop can be estimated with the Seinhorst function, a mathematical model that describes the relationship between the initial nematode population density and the yield (Schomaker & Been, 2003). Although Olthof & Potter (1973) noticed damage by *P. penetrans* on field-grown lettuce at 6000 *P. penetrans* (kg soil)⁻¹, they did not define a threshold density. The knowledge of this threshold is important to provide good management advice for lettuce growers.

The objective of this study was to investigate the population dynamics of *P. penetrans* in glasshouse-grown butterhead lettuce under normal cropping practices. Therefore, one commercial glasshouse without other important plant-parasitic nematodes was selected from the Belgian survey conducted in 2014. The nematode population was monitored at the commercial glasshouse during 2.5 years to gain insight into the factors influencing nematode densities. In addition, the damage threshold was estimated by growing lettuce in pots in the glasshouse and recording lettuce growth parameters over a wide range of *P. penetrans* population densities. The obtained knowledge will contribute to the set-up of integrated pest management tools and the reduction of chemical soil disinfestation.

Materials and methods

SPATIAL AND TEMPORAL DYNAMICS OF *P. PENETRANS* IN GLASSHOUSE LETTUCE IN ROTATION WITH LEEK

A glasshouse where *P. penetrans* was found in relatively high densities during a survey from 2014 (701 *P. penetrans* (100 ml soil)⁻¹ in the 0–30 cm soil layer and 143 *P. penetrans* (100 ml soil)⁻¹ in the 30–60 cm layer)

was selected for this study since no other important plant-parasitic nematodes were observed. The lettuce grower followed a rotation pattern consisting of black fallow in winter, cultivation of leek (*Allium porrum*) from March until June and two crops of butterhead lettuce. Every year or every other year, chemical soil disinfestation was applied in summer between the lettuce crops. The soil texture in the glasshouse was a fine sand with 5.1% of carbon, a pH-KCl of 5.8, 38 kg NO₃⁻ ha⁻¹, 12 kg NH₄⁺ ha⁻¹, 2333 mg P₂O₅ and 480 mg K₂O (l soil)⁻¹, according to a soil analysis of January 2016. The nutrient status of the soil was adjusted regularly by applying fertilisers as recommended for each crop and was based on yearly soil analyses.

The study was carried out on a plot where reduced lettuce growth caused by *P. penetrans* had been observed previously. Eight composite soil samples were taken after each harvest of lettuce and leek, chemical soil disinfestation and black fallow. Samples were taken at four spots (= replications) at every sampling event. A grid of one square metre (0.75 m × 1.4 m) was positioned on the same spot at every sampling occasion and two samples of ten cores each were collected per grid; one from 0–30 cm depth using a 2.5-cm diam. soil auger and one from 30–60 cm depth using a 3.5-cm diam. soil auger. This resulted in sample sizes of 1.47 and 2.88 l, respectively. In addition, eight randomly chosen leek plants with roots were harvested on each of the four grids 6 days before the last soil sampling (21 June 2018). At each sampling event, the grower was interviewed about the performance of the previous crop. Soil temperature was measured, except for the first sampling event. Samples were stored at 12°C and processed within 4 weeks.

The soil of each sample was thoroughly mixed and a subsample of 200 ml was taken. The organic fraction was separated from the mineral fraction by washing the soil through a 850 µm sieve (Chen *et al.*, 2000). The organic fraction, collected on top of the sieve and containing mainly small root fragments, was mixed in tap water for 1 min with a Waring blender at high speed setting and added to the mineral fraction in a 1 l beaker. At the final sampling event, leek roots were sampled separately from soil samples. Those leek roots with adhering soil were cut in 1 cm pieces and a 10 g subsample was mixed in tap water for 1 min, and washed over a 850 µm sieve into a 1 l beaker. The nematodes were extracted using the automated zonal centrifuge (Hendrickx, 1995) programmed for processing half of the volume, corresponding to 100 ml soil or 5 g roots. The obtained nematode suspension was collected in a glass

beaker and subsequently transferred to a counting dish. Total numbers of *P. penetrans* and non-plant-parasitic nematodes were determined using a binocular microscope (50 $\times$).

Nematode counts taken before planting and at harvest of the different crops represented the initial and final population density, respectively. These counts were used to determine the influence of the crops on the nematode population in the soil. If the data were normally distributed and variances were equal, the initial and final population density were compared using the Student's *t*-test. If those assumptions were not fulfilled, the Wilcoxon test was applied. Spearman's correlation was calculated to find out to what extent the numbers of *P. penetrans* were related to those of non-plant-parasitic nematodes. The strength and direction of the correlation between nematode numbers in the two soil layers was also examined by calculating Spearman's coefficient, as was the influence of temperature on nematode numbers. The statistical tests were performed at a confidence level of $P = 0.05$.

DAMAGE THRESHOLD FOR *P. PENETRANS* ON BUTTERHEAD LETTUCE

Two pot experiments were carried out during two different seasons. The first experiment was conducted in autumn with butterhead lettuce 'Brighton' and a second experiment in summer conditions with 'Cosmopolia'.

Multiplication of *P. penetrans*

Pratylenchus penetrans was multiplied on carrot discs (O'Bannon & Taylor, 1968) and in pots on maize (Teklu *et al.*, 2016) to ensure sufficient nematode numbers for the experiments. The population was originally collected from maize roots in Kinrooi (Belgium) and had been maintained on carrot discs since 2006. Culturing methods were slightly adapted from the original descriptions in the following ways. For the carrot disc method, carrots were washed with tap water, surface-sterilised with ethanol, peeled and cut in slices of 5–10 mm with a sterile knife in a laminar flow. Vermiform stages of *P. penetrans* were sterilised in 2000 ppm streptomycin sulphate overnight and washed three times with sterile water before inoculating them on the carrot discs (20–40 nematodes disc⁻¹). The carrot discs were maintained in Petri dishes and incubated in the dark at 21°C for 3 months. After incubation, the discs were cut into pieces and placed on a Baermann funnel in the mist chamber to collect the nematodes.

Five maize seeds ('Koloris' and 'LG3220') were seeded in 6 l pots filled with 5610 g silver sand on a layer of 390 g hydro grains. After 2 weeks, pots were infested at a rate of 8 *P. penetrans* (g dry soil)⁻¹ in four holes between the plants. Maize was grown for 3 months at 20°C day/16°C night and 16 h light. Plants were watered and fertilised three times a week. At harvest, roots were carefully washed with tap water, cut in 1 cm pieces and placed on a Baermann funnel in a mist chamber. Nematodes were collected regularly from carrot discs and maize roots until sufficient inoculum of *P. penetrans* was obtained. The nematodes were stored at 4°C until being used in the experiments.

Pot experiments

Pots of 17 cm diam. and 16 cm height were filled with 1.6 l heat-sterilised field soil (6% clay, 20% loam, 74% sand and 1.4% organic carbon) in both experiments. Lettuce was sown in peat blocks (1 seed per block of 5 $\times$ 5 $\times$ 5 cm³), provided by the practical research stations PCG and Inagro, and were transferred to the pots when plants reached the 4–6 leaf stage. The peat blocks with seedlings were in a 2 cm deep hole made in the sandy soil. This took place in autumn (5 October 2016) and spring (21 March 2018) for 'Brighton' and 'Cosmopolia', a popular winter and summer cultivar, respectively.

One day after lettuce planting, the soil in the pots was inoculated with nematode densities of 0, 50, 100, 200, 400, 800, 1600, 3200, 6400 and 12 800 nematodes (100 ml soil)⁻¹. Separate suspensions were made for every nematode density, so that an identical volume (80 ml) could be added to every pot. The inoculum was pipetted in four holes around each plant, each 5 cm deep. Immediately after applying the nematode suspension to the soil, the holes were carefully closed and plants were watered. Five replications for every density were used, except for the highest density where a shortage of inoculum only allowed four and three replicates with 'Brighton' and 'Cosmopolia', respectively.

Pots were organised in the glasshouse according to a randomised block design. Plants were watered and fertilised as needed and no pesticides were applied. The experiment with 'Brighton' was carried out during autumn (October–November 2016) at PCG, Kruishoutem, where pots were kept in a commercial glasshouse setting. Hence, the temperature fluctuated according to the season and was on average 15–20°C in the first part of the experiment and 10–15°C in the second part. Plants were harvested 8 weeks after planting. The experiment with the summer cultivar 'Cosmopolia' was performed in a glasshouse at

ILVO, Merelbeke, under controlled conditions simulating summer: day and night temperatures were 20 and 16°C, respectively, with 16 h light. These plants were harvested 6 weeks after planting.

Nematode damage was evaluated by measuring lettuce head and root weight, and scoring head colour, head development and root quality on a scale from 1-9. The head colour scale ranged from score 1, very dark, indicating a stressed plant, to score 9, normal light green colour, for a healthy crop. The head development refers to the head shape, a very important parameter for the quality of the produce. When the head is open and hollow, score 1 is given, while for a qualitative closed and filled head score 9 is given. Root quality was also scored on a scale from 1 (very bad root quality, roots break easily, several root lesions) to 9 (very good root quality, no root lesions).

The final nematode population was determined for the organic and mineral fraction in each pot. The entire root system was carefully removed from the soil, rinsed with water and cut into 1 cm pieces. The soil from the pot was mixed and a 200-ml subsample was taken for nematode extraction. Root and soil samples were processed as described above. Adults and juveniles of *P. penetrans* were counted in the total nematode suspension, except where there were high numbers when a subsample of at least 200 nematodes was counted.

STATISTICAL ANALYSIS AND MODELLING

Lettuce quality as well as nematode reproduction was described as a function of the applied initial nematode densities (P_i), using the models developed by Seinhorst (1966, 1986, 1998).

Lettuce quality

Measurements, directly or indirectly related to the quality of the lettuce heads (head development, head colour, head weight, root quality and root weight) were averaged for every initial nematode density (P_i). The Seinhorst model with $Z = 0.95$ was fitted to the averaged data (Seinhorst, 1986, 1998).

$$y = m + (1 - m) \times 0.95^{P_i/T-1} \quad \text{for } P_i > T$$

$$y = 1 \quad \text{for } P_i \leq T \quad (1)$$

The parameters tolerance limit (T) and relative minimum yield (m) were estimated for all measurements.

Population dynamics

The P_f (final population) for every initial nematode density (P_i) was log transformed, averaged over all repli-

cations and then back transformed. The maximum multiplication rate (a) and the maximum population density (M) for each experiment were estimated by modelling the relationship between P_i and P_f . The equation for migratory nematodes with more than one generation per growing period was used (Seinhorst, 1966; Schomaker *et al.*, 2013):

$$P_f = M \times P_i / (P_i + M/a) \quad (2)$$

All datasets were analysed using R studio and run in R-version 3.5.0. The goodness-of-fit of models was expressed as the coefficient of determination (R^2). Statistical differences between the estimated parameters of the two experiments were analysed using the least significant difference (LSD) test at $P = 0.05$.

Results

THE OCCURRENCE OF *P. PENETRANS* IN GLASSHOUSE LETTUCE IN ROTATION WITH LEEK

Different cultivars of butterhead lettuce were grown in rotation with leek in a commercial glasshouse during the 2.5 years of the study (Fig. 1). No above-ground symptoms indicating nematode damage were observed in any of the crops. The first soil samples were taken early in December 2015 after lettuce 'Gardia' (Fig. 1). They contained only few *P. penetrans*, most residing in the 30-60 cm soil layer ($10 (100 \text{ ml soil})^{-1}$) and about 500 non-plant-parasitic nematodes ($100 \text{ ml soil})^{-1}$ (Fig. 2A, B). Chemical soil disinfestation with 1,3-dichloropropene had been applied in June 2015 before the start of the observations and was applied again in August 2016. Immediately after removal of the plastic, 3 weeks after the nematicide treatment, *P. penetrans* was absent in the 0-30 cm layer and only 7 *P. penetrans* ($100 \text{ ml soil})^{-1}$ were found in the 30-60 cm layer, a population decrease of 59%. In addition, 93 and 90% of the non-plant-parasitic nematodes were killed due to this application in the higher and lower soil layer, respectively. However, the number of non-plant-parasitic nematodes recovered better after one cropping of butterhead lettuce compared with the numbers of *P. penetrans* (Fig. 2B).

Every winter there was a 3-month period of black fallow during which the population of *P. penetrans* remained more or less the same or increased slightly (Fig. 2). In 2016, the increase was mainly observed in the lower layer (from 10 to 85 *P. penetrans* ($100 \text{ ml soil})^{-1}$), while in 2018 the numbers of *P. penetrans* increased in the upper soil layer (from 27 to 66 *P. penetrans* ($100 \text{ ml soil})^{-1}$). Many

Fig. 1. Timeline of the crops grown in the commercial glasshouse starting in June 2015. The black dots represent the sampling time points. The green line represents the cultivation of butterhead lettuce with different cultivars (Ga = 'Gardia', Co = 'Cosmopolia', Lu = 'Lucrecia' and Pre = 'Presteria'). The blue line represents the cultivation of leek 'Krypton'. SD = chemical soil disinfestation.

juveniles were observed in the samples after each period of black fallow.

Leek 'Krypton' was grown every year from March until the end of June. Its influence on the soil population density of *P. penetrans* was investigated by comparing the population densities in the soil before planting (P_i) and immediately after harvest (P_f) in both soil layers (Fig. 3A). In 2016, the population density decreased significantly in the lower soil layer. The densities of *P. penetrans* were too low to notice any changes in 2017 (Figs 2, 3A). In 2018, an increase was noticed in both soil layers but was not statistically significant. In 2018, roots were also investigated for the presence of *P. penetrans*. Elongated brown necrotic root lesions of 0.5 cm could be observed and 5 g of roots contained 1741 ± 608 vermiform stages of *P. penetrans* and 2188 ± 700 eggs ($n = 4$).

In general, growing butterhead lettuce resulted in no or just a slight increase in the numbers of *P. penetrans* in the soil (Fig. 3B). The only significant increase in nematode population density was observed in the 30-60 cm layer after growing butterhead lettuce 'Cosmopolia' in the summer of 2016. As 'Gardia' was grown after chemical soil disinfestation, not enough *P. penetrans* were left in the soil to notice differences in root-lesion nematode population densities. The numbers of *P. penetrans* increased, although not significantly, when growing 'Lucrecia' in the summer of 2017, and 'Presteria' in autumn 2017.

Overall, a weak linear correlation was found between the numbers of *P. penetrans* in the 0-30 cm layer and those in the 30-60 cm layer ($R = 0.62$, $P < 0.01$, Spearman correlation). This was also the case for the numbers of non-plant-parasitic nematodes ($R = 0.64$, $P < 0.01$, Spearman correlation). No correlation was found between the densities of *P. penetrans* and the densities of non-plant-parasitic nematodes ($R = 0.28$, $P > 0.05$, Spearman correlation), averaged over the 0-60 cm layer. Furthermore, there was no correlation between the population density of *P. penetrans* in the 0-60 cm layer and the ambient temperature ($R = 0.01$, $P > 0.05$, Spearman correlation) (data not shown).

DAMAGE THRESHOLD FOR *P. PENETRANS* ON BUTTERHEAD LETTUCE

Two pot experiments were carried out to determine the damage threshold for *P. penetrans* on lettuce. A first experiment was set up in autumn with 'Brighton' and a second in summer conditions with 'Cosmopolia'. The lettuce plants grew for 8 and 6 weeks in the first and second experiment, respectively.

Weight and quality of lettuce heads

The lettuce head weight ranged between 191 and 266 g and 95 and 147 g for 'Brighton' and 'Cosmopolia', respectively. Using the Seinhorst equation (1), a relative minimum weight (m_w) of 0.12 and 0.85, and a maximum yield ($Y_{\max(w)}$) of 249 g and 132 g were calculated for the two cultivars (Table 1). The tolerance limit (T_w) was 3834 *P. penetrans* (100 ml soil) $^{-1}$ for 'Brighton' in autumn and 669 *P. penetrans* (100 ml soil) $^{-1}$ for 'Cosmopolia' in summer conditions. No significant differences could be detected for the relative minimum weight and the tolerance limit between both cultivars.

Lettuce head development is of importance for the quality of the crop. A decline in quality could be observed with increasing numbers of *P. penetrans* in the soil (Fig. 4). The tolerance limit (T_D) for the head development was 185 and 1314 *P. penetrans* (100 ml soil) $^{-1}$ for 'Brighton' and 'Cosmopolia', respectively. The relative minimum scores for head development (m_D) were 0.79 and 0.39; the maximum scores for head development ($Y_{\max(D)}$) were 6.23 and 7.64 (Table 2). No differences in colour of the lettuce heads could be noticed in pots with different densities of *P. penetrans*, for both experiments (data not shown).

Root weight and quality

Root weight and quality were measured as they affect head weight and development. The root weight ranged from 3.4 to 9.5 g and from 3.6 to 19.2 g for 'Brighton' grown in autumn and 'Cosmopolia' grown in summer conditions, respectively. An inverse relation was observed

Fig. 2. A: Number of *Pratylenchus penetrans* (100 ml soil)⁻¹ in the 0-30 and 30-60 cm soil layer. B: Number of *P. penetrans* and non-plant-parasitic nematodes (100 ml soil)⁻¹ averaged over the 0-60 cm soil layer and the ambient soil temperature over a period of 2.5 years. Day 1, the first sampling date, is 4 December 2015. Growing lettuce and leek is represented by the colours green and blue, respectively; white strips indicate black fallow. SD = chemical soil disinfestation. Error bars represent the standard error (n = 4).

Fig. 3. The initial (P_i) and final (P_f) population density of *Pratylenchus penetrans* before and after growing A: Leek, B: different butterhead lettuce cultivars in the 0-30 and 30-60 cm soil layer. Error bars represent the standard error ($n = 4$). An asterisk (*) shows significant differences between P_i and P_f ($P = 0.05$).

between root weight and nematode population density (Fig. 4). The relative minimum root weight (m_{RW}) was 0.76 and 0.51 g and the tolerance limit (T_{RW}) was 262 and 400 *P. penetrans* (100 ml soil)⁻¹ for 'Brighton' and 'Cosmopolia', respectively. The maximum root weight ($Y_{\max(RW)}$) was 6.25 g for 'Brighton' and 11.14 g for 'Cosmopolia' (Table 3). Additionally, root quality was affected by *P. penetrans* population density. The maximum root quality scores ($Y_{\max(Q)}$) and the relative minimum root quality scores (m_Q) were similar for 'Brighton' and 'Cosmopolia': around 8 for $Y_{\max(Q)}$ and 0.6 for m_Q (Table 4).

However, there was a significant difference between the tolerance limits in both experiments; T_Q was 48 and 204 *P. penetrans* (100 ml soil)⁻¹ for 'Brighton' and 'Cosmopolia', respectively.

Population dynamics

The relation between P_i and P_f was very well described with the model for population dynamics (2) in both experiments ($R^2 = 0.96$ and 0.99 ; Table 5). 'Brighton' and 'Cosmopolia' are both poor host for *P. penetrans*, because a decrease of the population was observed for every initial population density tested (Fig. 5). The maximum multi-

Table 1. Parameter values for the Seinhorst equations for the relation between initial population density (P_i) of *Pratylenchus penetrans* and lettuce head weight (g) according to the equation: $Y_W = m_W + (1 - m_W)0.95^{P_i/T_W - 1}$ for $P_i > T_W$ and $Y_W = 1$ for $P_i \leq T_W$.

Cultivar	T_W	m_W	$Y_{\max(W)}$	$SE_{T(W)}$	$SE_{m(W)}$	$SE_{Y_{\max(W)}}$	R^2	df	$LSD_{T(W)}$	$LSD_{m(W)}$
'Brighton'	3834	0.12	248.69	2517	0.83	1.78	0.69	7	–	–
'Cosmopolia'	669	0.85	131.83	1178	0.19	1.73	0.46	7	5960	1.83

P_i and the tolerance limit (T) are expressed in *P. penetrans* (100 ml soil)⁻¹, while yield (y) and relative minimum yield (m) are proportions and the maximum yield ($Y_{\max}$) is expressed in g. Parameter values of 'Brighton' grown in autumn were compared with those of 'Cosmopolia' grown in summer conditions using the LSD test. An asterisk (*) shows significant differences ($P = 0.05$). SE = standard error, df = degrees of freedom.

Table 2. Parameter values for the Seinhorst equations for the relation between initial population density (P_i) of *Pratylenchus penetrans* and lettuce head development according to the equation: $Y_D = m_D + (1 - m_D)0.95^{P_i/T_D - 1}$ for $P_i > T_D$ and $Y_D = 1$ for $P_i \leq T_D$.

Cultivar	T_D	m_D	$Y_{\max(D)}$	$SE_{T(D)}$	$SE_{m(D)}$	$SE_{Y_{\max(D)}}$	R^2	df	$LSD_{T(D)}$	$LSD_{m(D)}$
'Brighton'	185	0.79	6.23	202	0.09	0.25	0.36	7	–	–
'Cosmopolia'	1314	0.39	7.64	1741	0.76	0.22	0.43	7	3759	1.65

P_i and the tolerance limit (T) are expressed in *P. penetrans* (100 ml soil)⁻¹, while yield (y) and relative minimum yield (m) are proportions and the maximum yield ($Y_{\max}$) is expressed as a score between 1 and 9. Parameter values of 'Brighton' grown in autumn were compared with those of 'Cosmopolia' grown in summer conditions using the LSD test. An asterisk (*) shows significant differences ($P = 0.05$). SE = standard error, df = degrees of freedom.

Table 3. Parameter values for the Seinhorst equations for the relation between initial population density (P_i) of *Pratylenchus penetrans* and lettuce root weight (g) according to the equation: $Y_{RW} = m_{RW} + (1 - m_{RW})0.95^{P_i/T_{RW} - 1}$ for $P_i > T_{RW}$ and $Y_{RW} = 1$ for $P_i \leq T_{RW}$.

Cultivar	T_{RW}	m_{RW}	$Y_{\max(RW)}$	$SE_{T(RW)}$	$SE_{m(RW)}$	$SE_{Y_{\max(RW)}}$	R^2	df	$LSD_{T(RW)}$	$LSD_{m(RW)}$
'Brighton'	262	0.76	6.25	312	0.14	0.26	0.30	7	–	–
'Cosmopolia'	400	0.51	11.14	173	0.15	0.43	0.62	7	765	0.44

P_i and the tolerance limit (T) are expressed in *P. penetrans* (100 ml soil)⁻¹, while yield (y) and relative minimum yield (m) are proportions and the maximum yield ($Y_{\max}$) is expressed in g. Parameter values of 'Brighton' grown in autumn were compared with those of 'Cosmopolia' grown in summer conditions using the LSD test. An asterisk (*) shows significant differences ($P = 0.05$). SE = standard error, df = degrees of freedom.

Table 4. Parameter values for the Seinhorst equations for the relation between initial population density (P_i) of *Pratylenchus penetrans* and lettuce root quality according to the equation: $Y_Q = m_Q + (1 - m_Q)0.95^{P_i/T_Q - 1}$ for $P_i > T_Q$ and $Y_Q = 1$ for $P_i \leq T_Q$.

Cultivar	T_Q	m_Q	$Y_{\max(Q)}$	$SE_{T(Q)}$	$SE_{m(Q)}$	$SE_{Y_{\max(Q)}}$	R^2	df	$LSD_{T(Q)}$	$LSD_{m(Q)}$
'Brighton'	48	0.66	8.41	21	0.03	0.26	0.90	7	–	–
'Cosmopolia'	204	0.60	8.01	50	0.04	0.12	0.95	7	115*	0.11

P_i and the tolerance limit (T) are expressed in *P. penetrans* (100 ml soil)⁻¹, while yield (y) and relative minimum yield (m) are proportions and the maximum yield ($Y_{\max}$) is expressed as a score between 1 and 9. Parameter values of 'Brighton' grown in autumn were compared with those of 'Cosmopolia' grown in summer conditions using the LSD test. An asterisk (*) shows significant differences ($P = 0.05$). SE = standard error, df = degrees of freedom.

Fig. 4. The relation between the initial population density (P_i) of *Pratylenchus penetrans* and A: the relative weight of the lettuce head, B: the relative lettuce head development, C: the relative root weight and D: relative root quality for 'Brighton' grown in autumn and 'Cosmopolia' grown in summer conditions. A line was fitted according to the Seinhorst equation: $y = m + (1 - m)0.95^{P_i/T_y}$ for $P_i > T_y$ and $y = 1$ for $P_i \leq T_y$.

plication rate (a) of the *P. penetrans* population was 0.31 for 'Brighton' and 0.40 for 'Cosmopolia' (Table 5). The maximum population density (M) was 3609 and 13 090 *P. penetrans* (100 ml soil)⁻¹ for 'Brighton' and 'Cosmopolia', respectively.

Discussion

To obtain more insight into the factors influencing the dynamics of a *P. penetrans* population in a commercial lettuce glasshouse, nematode population dynamics were monitored during a 2.5 year period. In addition, we set up pot tests to determine the damage threshold densi-

ties of the root-lesion nematode for butterhead lettuce in summer and autumn croppings. Overall, numbers of *P. penetrans* were low in this study, which makes it hard to gain a deeper insight into factors influencing *P. penetrans* dynamics. Moreover, nematode numbers in the four replicated spots were highly variable. The annual use of the soil fumigant 1,3-dichloropropene most likely contributed to the low occurrence of *P. penetrans* (Zasada *et al.*, 2010). Although nematode numbers declined considerably, nearly half (41%) of the population in the 30–60 cm soil layer survived the application. This is not unexpected as it is known that a nematicide application cannot be 100% effective (Haydock *et al.*, 2006). The surviving nematodes can migrate to the upper soil lay-

ers when a new crop is planted and infect roots growing downwards, so they are a risk for future crops. Indeed, a slow return of the population in the 0-30 cm soil layer was noticed in the commercial glasshouse when sampling 1 year after the disinfestation. The mobility of *P. penetrans* depends on several factors, such as soil texture and water availability (Castillo & Vovlas, 2007). The sandy soil in the glasshouse we monitored and the regular water supply were conducive to the upwards migration of remaining *P. penetrans*.

It is known that black fallow in general has a negative effect on the population of *P. penetrans* (Pudasaini *et al.*, 2006; Viaene *et al.*, 2006). However, it was reported that at low population densities the number of nematodes can remain the same (Grabau *et al.*, 2017). Although low densities were observed in our study, the population

Fig. 5. The relation between the initial (P_i) and final (P_f) population density of *Pratylenchus penetrans* (roots and soil) for 'Brighton' grown in autumn and 'Cosmopolia' grown in summer conditions. A line was fitted according to the equation: $P_f = M \times P_i / (P_i + M/a)$ for population dynamics. The diagonal dashed line represents the population equilibrium line ($P_f = P_i$).

Table 5. Parameter values of the population dynamics model for the relation between initial (P_i) and final population density (P_f) of *Pratylenchus penetrans* (100 ml soil) $^{-1}$ for 'Brighton' grown in autumn and 'Cosmopolia' grown in summer conditions, according to the model $P_f = M \times P_i / (P_i + M/a)$.

Cultivar	M	a	SE_M	SE_a	R^2	df	LSD_M	LSD_a
'Brighton'	3609	0.31	1247	0.05	0.96	7	–	–
'Cosmopolia'	13 090	0.40	8180	0.03	0.99	7	1.43	0.35

Multiplication rate (a) is dimensionless while maximum population (M) is measured as *P. penetrans* (100 ml soil) $^{-1}$. Parameter values of 'Brighton' were compared with those of 'Cosmopolia' using the LSD-test ($P = 0.05$). SE = standard error, df = degrees of freedom.

* Significantly different at $P = 0.05$.

surprisingly increased sometimes after 3 months of black fallow in winter. This contradictory result can probably be explained by hatching, since a lot of juveniles were observed at the end of the fallow period. Hatching of *P. penetrans* is influenced by the host species, temperature (Pudasaini *et al.*, 2008) and soil moisture (Gaur & Haque, 1987). During winter the soil temperature in non-heated glasshouses is around 4-6°C at 10-15 cm depth, which still allows the development of *P. penetrans*. Mokrini *et al.* (2016) showed that the reproduction factor of different *P. penetrans* populations was 1.0-1.6 on carrot discs incubated for 8 weeks and even 2.7-3.7 after 12 weeks at 10°C. Dunn (1972) showed that *P. penetrans* can hatch even at 0-4°C. The lettuce grower only watered the soil at the end of black fallow. We assume that the change of moisture content stimulated hatching of *P. penetrans*.

Eggs in the nematode suspensions after extraction of the soil samples were not counted as those from *P. penetrans* cannot be distinguished from eggs of other nematode species. The number of eggs before and after leek, fallow and lettuce could have explained some of the observations made in this study, especially if the observed increase in number was due to hatching from eggs already present, or due to reproduction of *P. penetrans* on the plants.

The population of *P. penetrans* decreased or remained the same after growing leeks. This is in contrast to Koot & Kroonenbackbier (1999), who showed that leek is a good host for *P. penetrans*. At the last cropping cycle, besides taking soil samples, leek plants were also harvested and the roots were analysed for *P. penetrans*. Overall, 5 g roots contained 1741 (± 608) *P. penetrans*, which corresponds with 16 *P. penetrans* (100 ml soil) $^{-1}$ in the upper layer or 11% of the total soil population. Leek can be considered as a trap crop as the roots are removed from the soil at harvest. A growth reduction of leek was never observed, probably due to the low numbers in the glasshouse. Koot

& Kroonenbackbier (1999) observed a growth reduction of leek at 545 *P. penetrans* (100 ml soil)⁻¹.

Growing lettuce had a minor impact on the population of *P. penetrans* in the commercial glasshouse, even though lettuce is known as a good or maintenance host (Oostenbrink *et al.*, 1957; Moretti *et al.*, 1981). Moretti *et al.* (1981) showed that both temperature and cultivar play a role in the reproduction of *P. penetrans*. They observed a maximum population increase of 2.1 at 21–22°C with an initial population density of 250 *P. penetrans* (100 ml soil)⁻¹, whilst at 14–15°C reproduction was reduced but not inhibited. In addition to reproduction, the length of the life cycle of *P. penetrans* also depends on temperature and can take between 3 and 8 weeks. According to Castillo & Vovlas (2007) the life cycle of *P. penetrans* was 46, 38, 28 and 26 days at 17, 20, 25 and 27°C, respectively. In the current study the temperature measured in the glasshouse at sampling before and after the first lettuce crop in summer was around 20°C, while it was lower at the end of the second crop, indicating a life cycle duration of at least 38 days. Comparing soil temperatures with other Belgian glasshouses, the average soil temperature is estimated to be around 20°C and 13–14°C during the first and second lettuce crop, respectively. The first lettuce crop in the commercial glasshouse, ‘Cosmopolia’ grown in 2016, lasted 35 days and resulted in a small population increase. The other lettuce cultivars in the commercial glasshouse with growing periods of 48 days for the first crop and 91–95 days for the second crop did not affect the population density. In our pot experiments, summer conditions were simulated with a temperature of 16–20°C for ‘Cosmopolia’, but a decrease in *P. penetrans* numbers was observed with a maximum multiplication rate of 0.41. The plants were grown for 42 days, barely enough to complete a life cycle at 16–20°C (Castillo & Vovlas, 2007). Similarly, the population in the pot experiment during autumn with ‘Brighton’, lasting 56 days at 20°C and lower, had a maximum multiplication rate of 0.31. The short periods that lettuce plants remain in the field could explain the small or lack of increase in *P. penetrans*. Lettuce grown outside in the field resulted in an increase of the population of *P. penetrans* in summer and even in autumn (Olthof & Potter, 1973, 1974). This lettuce was planted in infested soil and grown for 57 days in summer (around 23°C) and 75 days in autumn, respectively which allowed the completion of a life cycle. In addition, crisphead lettuce was used instead of butterhead lettuce, which can also influence reproduction.

Another reason for the lack of reproduction in the pot experiments could be the selected *P. penetrans* popula-

tion. Pot tests were conducted with the Kinrooi population, originating from maize and cultured on carrot discs for more than 13 years. Tests with another population (PSKW), originating from lettuce and cultured on carrot discs and maize for only 2 years, did not result in different lettuce weights compared to Kinrooi (data unpubl.). However, the final populations of PSKW on lettuce ‘Cosmopolia’ were the same as the initial population, *i.e.*, higher than those obtained with the Kinrooi population. The populations used in the studies of Olthof & Potter (1973, 1974) and Moretti *et al.* (1981) were cultured on different hosts, including lettuce. Differences in reproduction and pathogenicity between *P. penetrans* populations have already been reported (Olthof, 1968; France & Brodie, 1995; Castillo & Vovlas, 2007) and could also explain the failure of *P. penetrans* to reproduce in our pot experiments. In addition, the extraction efficiency of the automated zonal centrifugation machine is not 100% and eggs are not counted, so the real population might be 10% higher than assumed.

Often the whole nematode community is used as a bio-indicator to evaluate cultivation strategies or crop types (Neher & Olson, 1999; Berkelmans *et al.*, 2003; van Diepeningen *et al.*, 2006; Quist *et al.*, 2016). Large fluctuations in the number of non-plant-parasitic nematodes in the samples were observed during the monitoring but no link with the *P. penetrans* population was found. We hypothesised that soil fumigation would result in a significant reduction of beneficial nematodes and this could have been an explanation of the expected increase of *P. penetrans* (Timper *et al.*, 2012). However, we observed a relatively fast rebound of the non-plant-parasitic nematodes after an initial decline immediately following fumigation but no considerable increase in *P. penetrans*. This could lead to the conclusion that an assumed competition between non-plant-parasitic nematodes and *P. penetrans* remained the same before and after chemical disinfection, and that *P. penetrans* is suppressed by certain micro-organisms or other types of nematodes. We did not identify the composition of the nematode or the microbial community, so we were unable to check this hypothesis. However the explanation can also be found in the simple fact that *P. penetrans* needs time to build up its population, unlike the bacterial-feeding nematodes, and that regular application of nematicides in the commercial glasshouse we monitored does not allow this. In addition, lettuce and leek are slowing down the population build-up in the soil. Similar observations were made in a field study after application of 1,3-dichloropropene where it took years for the plant-parasitic nematodes *Rotylenchus robustus*, *Tri-*

chodorus primitivus and *Paratrichodorus pachydermus* to reach their pre-fumigation densities (Boag & Alphey, 1988). A more thorough investigation of the nematode community could be informative to work out relations between crop management practices and *P. penetrans*.

There are no reports of the damage threshold for *P. penetrans* in butterhead lettuce determined with the Seinhorst model. For crisphead lettuce, the damage threshold for *Meloidogyne hapla* was estimated to be 100–200 eggs (100 ml soil)⁻¹ in microplots, and 700–800 eggs (100 ml soil)⁻¹ in pots (Viaene & Abawi, 1996). In our study, the damage threshold for the crop weight was 669 and 3834 *P. penetrans* (100 ml soil)⁻¹ in pots in summer and autumn conditions, respectively. Outside-grown transplanted crisphead lettuce showed losses in lettuce yield of 36 and 27% at initial population densities equivalent to 840 *P. penetrans* (100 ml soil)⁻¹ in summer and autumn, respectively (Olthof & Potter, 1973, 1974). In our study the damage threshold for the autumn-grown lettuce was much higher than the other reported thresholds but it was quite similar for the summer crop. Our pot experiments were conducted in heat-sterilised soil by which secondary infections that could have been present in the field in the autumn experiment were avoided. This might explain the fact that higher nematode populations were necessary to obtain yield losses in our pot test, next to the possible influence of cultivar, soil and climatic conditions. Furthermore, the standard errors for the calculated parameter values were very high and therefore the estimated damage thresholds should be interpreted with caution. This high variability is due to errors estimating P_i and yield; therefore, more repetitions should be included. Next to that, pot tests are indicative and field tests should be carried out to validate these results. For both pot tests, the damage thresholds for root weight and root quality were lower than those for crop weight, which means that damage on roots does not immediately result in lower crop weight.

No damage was observed on lettuce during the monitoring of the commercial glasshouse, probably due to the low densities of *P. penetrans* (≤ 124 *P. penetrans* (100 ml soil)⁻¹). However, in the summer of 2018, the grower observed growth reduction in another glasshouse, where 220 *P. penetrans* (100 ml soil)⁻¹ and 323 *P. penetrans* (5 g roots)⁻¹ were counted in a soil sample taken after damage was observed. Furthermore, in the preliminary survey on lettuce, damage was found at a final population density of 701 *P. penetrans* (100 ml soil)⁻¹. In commercial lettuce production, heads should reach 450 g to be marketable. Plants in the pot experiments had maximum weights of

266 g and 147 g in the autumn and summer crop, respectively. Therefore, it is strongly recommended to consider the damage thresholds calculated for root quality when making management decisions, because healthy roots will ensure a healthy lettuce head.

The damage threshold for *P. penetrans* can vary a lot between vegetables. The damage thresholds for *P. penetrans* for faba bean and carrot were 6.2 (g soil)⁻¹ and 1.37–1.88 (g dry soil)⁻¹, respectively (Seinhorst, 1998; Teklu *et al.*, 2016). These numbers are equivalent to 868 and 192–263 *P. penetrans* (100 ml soil)⁻¹. Other studies showed damage at 50 *P. penetrans* (100 ml soil)⁻¹ for bean; 0.45 *P. penetrans* (g soil)⁻¹ (equivalent to ca 63 (100 ml soil)⁻¹) for Brussels sprouts, cucumber, eggplant and tomato; 666 *P. penetrans* (kg soil)⁻¹ (equivalent to ca 93 (100 ml soil)⁻¹) for onion; 6000 *P. penetrans* (kg soil)⁻¹ (equivalent to 840 (100 ml soil)⁻¹) for cabbage, cauliflower, potato and 18 000 *P. penetrans* (kg soil)⁻¹ (equivalent to 2520 (100 ml soil)⁻¹) for beet and spinach (Olthof & Potter, 1973, 1974; Miller, 1978; Elliott & Bird, 1985). Our calculated damage thresholds are comparable with those for cabbage, cauliflower, potato, beet and spinach.

In conclusion, this study revealed that *P. penetrans* is a minor problem in glasshouse-grown lettuce but one that should not be overlooked. The population densities must be kept below 48–204 *P. penetrans* (100 ml soil)⁻¹ to secure a good root quality, necessary to obtain a marketable crop. However, other plant pathogens can also influence lettuce yield, so regular monitoring for pathogens as well as nematodes is highly recommended. Combining black fallow with a rotation of leek and lettuce seems to be a good practice to keep the nematode population low for at least 21 months. However, critical nematode densities might be achieved over time, requiring measures that reduce numbers effectively, such as chemical soil disinfection. Lettuce growers should be aware that this management strategy does not kill all the nematodes and that the population of *P. penetrans* will recover slowly.

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