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Population dynamics of the potato cyst nematode, *Globodera pallida*, in relation to temperature, potato cultivar and nematicide application

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The impact of increasing temperatures on the population dynamics of the soil-dwelling nematode *Globodera pallida*, a persistent and economically important pest of potatoes, was investigated. The reproductive factor (final population/initial population) and length of life cycle were found to be temperature sensitive. Pot experiments performed over 4 months allowed comparison of the effect on development of *G. pallida* of two temperature regimes: an average temperature comparable to current field conditions (14.3 °C) and an average temperature above current field conditions (17.3 °C). A larger second generation of juveniles was observed at 17.3 °C compared to 14.3 °C. Multiplication of *G. pallida* at field sites in Shropshire and East Lothian (average soil temperatures of 14.1 and 15.1 °C, respectively, during potato cropping) was also examined. A quantitative PCR assay and visual examination of roots were used to monitor the dynamics of the *G. pallida* populations in both field sites at 4-weekly intervals. Four cultivars, Desirée, Cara, Maris Piper and Estima, were grown with and without nematicide treatments. Nematicide treatments suppressed population increases at both sites. Females were observed on the roots of cvs Cara and Desirée at the end of the

growing season in Shropshire, but not at East Lothian, and are likely to represent a second generation.

Keywords: population dynamics, potato cyst nematode, second generation, temperature, nematicide treatment

Introduction

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* are major parasites of potatoes and other members of the Solanaceae family. Infected plants typically have a smaller root system (Trudgill & Cotes, 1983), have a diminished capacity to take up nutrients from the soil and are adversely affected by water stress or disturbance of nutrient metabolism. According to the EPPO Global Database (2019), *G. rostochiensis* and *G. pallida* have been detected in 72 and 49 countries respectively. In the UK, the direct and indirect crop losses caused by PCN were valued at 9% of yield annually (Evans, 1993) and the economic cost of PCN to the UK processing and fresh market potato industry was estimated at *c.* £26 million in 2009 (Twining *et al.*, 2009). Increasing management costs after implementation of the PCN Directive (2007/33/EU), which requires improved pre-planting soil testing for all seed crops and an annual survey of 0.5% of ware land, also add to the economic impact of PCN. In fields where PCN are detected, the directive prohibits the growing of seed potatoes, and ware potatoes may only be grown under an officially approved control programme that includes using resistant varieties, nematicides or other control measures (Hockland *et al*., 2000).

The population dynamics of *G. pallida* and *G. rostochiensis* and associated yield loss of the potato crop differ greatly among years and locations (Greco *et al.*, 1982; Seinhorst,

1982). As mentioned above, damage caused by PCN, particularly *G. rostochiensis*, can be minimized by the selection of resistant potato cultivars within a rotation cycle (Trudgill, 1986). However, with susceptible cultivars, factors including the amount of the initial population of PCN (Trudgill *et al.*, 2014), environmental factors (Jones, 1983) and soil type (Trudgill, 1986; Elston *et al.*, 1991) can affect multiplication rates. In general, the larger the population of *Globodera* spp. in the field at planting, the greater the yield losses (Trudgill *et al.*, 2014; Seinhorst, 1982), although it is also known that PCN reproduction is density dependent (Trudgill *et al.*, 2014) and at lower densities the multiplication rate is higher.

In the last century, global temperatures have been increasing due to climate change and the effect of this trend on crop losses due to plant pests and diseases, including soil dwelling organisms such as PCN, is of concern (Ellenby & Smith, 1975; Stanton & Sartori, 1990; Munir *et al.*, 2009, Ebrahimi *et al.*, 2014; Jones *et al.*, 2017; Skelsey *et al.*, 2018). Recently Jones *et al.* (2017) and Skelsey *et al.* (2018) have reported on the potential impact of climate change in the UK on the potato cyst nematodes *G. rostochiensis* and *G. pallida* based on experiments conducted in controlled environments. While the reports differ in some respects, they generally agree that increases in soil temperatures from current levels are likely to lead to increased multiplication of PCN, particularly *G. rostochiensis*. *Globodera pallida* is more adapted to cooler temperatures than *G. rostochiensis* and, hence, increases in soil temperatures above current levels are more likely to benefit the latter species. However, the relationship between temperature and population dynamics for *G. pallida* has not been examined under different *in vivo* field conditions in the UK. The aim of the present study was to investigate the multiplication and development of PCN at two field sites in the UK and compare this with multiplication and development in controlled environments.

Materials and methods

Nematodes and plant material

Globodera pallida population (Lindley) (Pa2/3) cysts from The James Hutton Institute (JHI) PCN collection were used for experiments in temperature-controlled conditions. This population had been maintained in glasshouse conditions (20 °C 16 h, 16 °C, 8 h) for over 30 years at the JHI. Cysts were collected on a 250 µm sieve, randomly selected and packed into nylon bags containing 30 cysts per bag. Cysts of *G. pallida* (Lindley) were also used for the validation of qPCR assays.

The susceptible cultivar Desirée and the moderately resistant cultivar Morag, which has resistance to *G. pallida* derived from *Solanum vernei* (Phillips & Trudgill, 1998), were used in a pot experiment to examine the life cycle of *G. pallida* in temperature-controlled conditions. For the field experiment, four susceptible cultivars were used at each site (Desirée, Maris Piper, Cara and Estima) whilst the susceptible cultivar Edzell Blue, which produces blue tubers, was used to produce guard rows as an additional measure to distinguish the boundaries of the plots. The four cultivars selected are commonly grown in the UK and are classified as early (Estima), main crop (Desirée and Maris Piper) and late cropping (Cara).

Occurrence of juveniles, males and cysts in the soil under different temperature regimes in controlled environments

Growth cabinets were used to monitor the occurrence of hatched juveniles, free-living adult males and new cysts of *G. pallida* in the soil at two different temperature regimes. The growth cabinets (Phytotron model 1700; Sanyo) were set as follows: (i) 16 °C for 16 h with

light (525 µmol m^{-2} s^{-1}) and 11 °C for 8 h with no light and (ii) 19 °C for 16 h with light and 14 °C for 8 h with no light. Soil temperatures were recorded every 3 h in the pots in the growth cabinet experiment with DS1920-F5 Temperature ibuttons (HomeChip Ltd) placed into the soil at a depth of 10 cm. The average soil temperatures of these regimes were 14.3 and 17.3 °C, respectively. The relative humidity inside the growth cabinets was maintained at 75%. The accumulated day degrees (DD) were calculated using the formula $DD = (T_{mean} - T_{base}) \times Days$, where T_{mean} is the mean temperature of that day and T_{base} is the base temperature needed for *G. pallida* to hatch, which was 4 °C according to Ebrahimi *et al.* (2014).

Tuber pieces (3 cm diameter) with a single sprout were cut with a melon scoop from tubers of cvs Desirée or Morag and planted in pots containing 500 g autoclaved sand:loam (50:50). Plants (96 per cultivar) were grown in the greenhouse until they were approximately 10 cm high before being moved to the growth cabinets. The plants were arranged in a randomized design in two growth cabinets for each temperature regime and, 2 days later, a nylon cyst bag containing 30 cysts was planted into the soil of each pot, at a depth of about 5 cm, beside the tuber piece. Five weeks after inoculation, the cyst bags were removed from the pots to distinguish between juvenile nematodes originating from these cysts and those from new females. The bags were removed with tweezers by pinching the top of the bag at the soil surface and pulling from the soil.

Three pots per temperature and cultivar were randomly selected at 7-day intervals for 16 weeks. The juveniles and males were extracted from the soil using the Baermann funnel method (Viglierchio & Schmitt, 1983). After 2 days, nematodes were collected and the numbers of juveniles and males were counted with a microscope. Additionally, the total number of cysts per pot was determined in weeks 8 to 16 by using acetone flotation to

117 recover the cysts that were collected in the sieves used with each Baermann funnel (Brodie *et*
118 *al*,, 1976).

119

120 **Field trials**

121 In 2011, field trials took place in two locations that were naturally infested by *G. pallida*:
122 East Lothian (lat 56.0170, long −2.8375) and a site near Harper Adams University, Newport
123 (lat 52.7797, long −2.4275). The soil temperatures were monitored with DS1920-F5
124 temperature monitoring ibuttons that were placed in the centre of two neighbouring potato
125 ridges at a depth of 20 cm. Temperatures were recorded every 3 h during the trials.

126 The treatment of 10% oxamyl nematicide granules (55 kg ha^{-1}) was applied at the
127 time of planting potatoes in East Lothian and fosthiazate (30 kg ha^{-1}) was applied
128 immediately before planting at the site near Harper Adams. The farm managers were
129 responsible for all agricultural operations, including nematicide application, fertilization and
130 application of other crop protection products at each site.

131 Each site had six experimental blocks and, within each, there were five replicates of
132 four cultivars (Desirée, Maris Piper, Cara and Estima) in a randomized design. One replicate
133 consisted of three tubers of each cultivar, planted 25 cm apart. Between trials and at the end
134 of each row, two tubers of the guard plants (Edzell Blue) were planted 25 cm from the
135 experimental blocks and from each other. The experiments were set up on 4 April 2011 and 4
136 May 2011 at the Harper Adams and East Lothian sites, respectively.

137 To estimate the initial populations of PCN, 500 g of soil was taken from each
138 experimental block with a 15 mL corer. Soil samples were subsequently collected at
139 approximately monthly intervals, from May to September at Harper Adams and from June to

October in East Lothian. During harvesting, approximately 5 L of soil, containing and surrounding the roots of each of the three plants per replicate, was collected with a spade, combined and mixed in a bucket. Subsequently, a subsample (at least 500 g) was placed in a labelled bag. Soil samples were air dried in the glasshouse by spreading each in a plastic tray (38 × 24 × 5 cm) and, when dry, a 400 g soil sample was packaged in a labelled paper bag and transferred to Science and Advice for Scottish Agriculture (SASA, UK) for cyst extraction using their automated soil washing carousel (Meku). The wet filter papers on which the cysts and debris were collected following their extraction from the soil with the carousel, were dried and the cysts were further purified by acetone flotation (Brodie *et al.*, 1976) and then transferred into a 2 mL Eppendorf tube384

To monitor nematode development, root samples of cvs Desirée and Cara were collected when each trial was harvested. The root systems for each replicate of three plants were cut off and the remaining soil was gently shaken off. Roots were chopped into 5–7 cm segments and stored in FAA (formalin–acetic–alcohol, 2:1:10) solution (Hooper, 1970). Later, a 0.5 ± 0.2 g subsample was stained with acid fuchsin (Bridge *et al*., 1981) and then examined by microscope (Olympus S7-ST).

DNA extraction from soil samples

The floats obtained following acetone purification were placed in 2 mL Eppendorf tubes with two metal beads per tube and pulverized in a mixer mill MM300 (Retsch) for 1.5 min at 30 Hz. The resulting powder was mixed with 0.5 mL GeneScan lysis buffer (Neogen Europe Ltd) and ground again for 30 s. Samples were centrifuged for 15 s at 15 600 ***g*** and then 5 µL

of 20 mg mL^{-1} proteinase K in 40% (v/v) glycerol (Sigma-Aldrich) was added and incubated for 1 h at 65 °C. After incubation, 0.5 mL chloroform:isoamyl alcohol (24:1) (Sigma-Aldrich) was added and mixed by inverting the tube five times. Samples were centrifuged for 10 min at 15 600 ***g*** in an Eppendorf centrifuge, the upper aqueous phase (450 µL) was transferred into a new tube and 360 µL of ice-cold propan-2-ol (VWR) was added and mixed thoroughly. Subsequently, samples were incubated for 30 min at 20 °C, centrifuged for 10 min at 15 600 ***g*** and the pellet was retained. The pellet was washed twice with 0.5 mL 75% ethanol and centrifugation for 5 min at 15 600 ***g*** and then resuspended in 100 µL sterile dH_2O.

The resulting DNA was further purified on PVPP columns. First, a 600 µL suspension of 10% PVPP (Sigma-Aldrich) was made up with sterile distilled water and transferred to a spin column (NBS Biologicals) in a 2 mL microcentrifuge tube. Tubes were centrifuged at 11 000 ***g*** for 1 min, the catch-tube was emptied and the centrifugation was repeated (the spin column was rotated 180° within the centrifuge before the second spin). The resuspended DNA was transferred to the spin column and centrifuged at 11 000 ***g*** for 1 min. Purified eluate was transferred to a new sterile 1.5 mL Eppendorf tube and stored at −20 °C.

PCN quantification by qPCR

The oligonucleotide design and optimization of specificity and sensitivity of the PCN qPCR assay was previously described by Reid *et al.* (2010). The primers and probes used were designed for the rDNA ITS1 region of *G. pallida* and *G. rostochiensis*; primer 1 (forward) 5′-CGTTTGTTGTTGACGGACAYA-3′, primer 2 (reverse) 5′-GGCGCTGTCCRTACATTGTTG-3′, *G. pallida* MGB probe 5′-6FAM-CCGCTATGTTTGGGC-3′, *G. rostochiensis* MGB probe 5′-6FAM-

CCGCTGTGTATKGGC-3′. All DNA from the field samples was tested for both PCN species; however, no *G. rostochiensis* was detected when compared to the DNA standard prepared from *G. rostochiensis* cysts from the JHI collection. To determine whether the assay was valid over a wide range of cyst densities, DNA was extracted from *G. pallida* (Lindley) cysts that had been recovered from a 1:1 sand:loam mixture (260 g per pot) with 12 to 644 cysts per pot (average number of eggs 205.4 ± 20.3 per cyst). DNA was extracted from these cysts as described for the field samples.

Real-time qPCR reactions were set up using a Genesis Workstation 150 (Tecan Inc.) in 96-well plates (Applied Biosystems/Thermo Fisher Scientific) at SASA, UK. The 30 µL reactions contained 15 µL Environmental BLUE Ready Mix (Eurogentec Ltd), 1.25 µL each of the forward and reverse primers for either species of PCN (5 pmol μL^{-1}), 1.25 µL of either the *G. pallida*- or *G. rostochiensis*-specific probe (5 pmol μL^{-1}), 6.25 µL distilled water (Sigma-Aldrich) and either 5 µL DNA that had been extracted from samples and diluted 1:10 with H_2O, or 5 µL H_2O as a negative control. The reactions were then aliquoted in triplicate into 384-well plates (Applied Biosystems) using a Genesis robot (Tecan). Standards (in triplicate) were created from *G. pallida* cyst DNA dilutions of 10, 1, 0.1, 0.01 and 0.001 ng μL^{-1}. Amplification was performed in an ABI 7900HT (Applied Biosystems) real-time machine run in the standard mode with the following cycling conditions: 50 °C for 2 min, 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Linear regression of the quantity of copies (qty values) versus egg number and calculation of the corresponding R^2 value were performed using EXCEL v. 16.0.4456.1003 (Microsoft) and GENSTAT v. 16.1.010916.

The average value of qty values obtained from the qPCR performed on DNA extracted from cysts was multiplied by 10 (for the dilution) and fitted to the validated linear regression model. In order to establish the number of eggs per g soil, the number of eggs

obtained from qPCR was divided by 400 g (dry weight of the soil sample from the field). For standardizing the egg numbers between plots and fields, the data is presented as a multiplication ratio of the population at the sampling time point divided by the initial number of eggs in that experimental plot.

Hatching of field cysts

Cysts were recovered from soil samples collected at the final harvest and subjected to a period of at least 3 months at 4 °C. Hatching experiments were performed on a thermal gradient table following the protocol of Kaczmarek *et al.* (2014a, b) with a temperature range from 5 to 19 °C.

Statistical analysis

Data was transformed and analysed using GENSTAT v. 17.1 and EXCEL v. 14.0.4760.1000 and v. 16.0.4456.1003. Data (numbers of J2s, males, cysts and eggs) were analysed using standard analyses of variance, linear and logarithmic regression as well as *t*-tests as detailed in the text. The data were subjected to logarithmic transformations where specified. The hatching data was analysed following the procedures in Kaczmarek *et al.* (2014a, b).

Results

Occurrence of juveniles, males, and cysts in the soil under two different temperature regimes

The first presence of juveniles in the soil was recorded at week 1 for both cvs Desirée and Morag and in both the 14.3 and 17.3 °C temperature regimes, after 62 and 80 DD respectively. This was followed by an increase in numbers and then a subsequent decrease after week 2 (between 104 and 295 DD; Fig. 1a,b). Around 1219 DD (week 10 at 17.3 °C), increasing numbers of J2s were recorded with Desirée (Fig. 1b) until the end of the experiment at week 16 (1500 DD), with comparable numbers (51 ± 23 J2 per pot) to those observed during the first hatching peak. A small and later second hatch was also observed at 14.3 °C at 1019 DD (week 14; Fig. 1a) with Desirée. Juveniles recovered from the pots with Morag, kept the same hatching trend as susceptible Desirée. The fewest numbers of juveniles were observed for Morag at the cooler temperatures.

The first appearance of males in both temperature regimes was at week 5 (332 DD) (Fig. 1c, d) with the highest numbers of males at 14.3 °C on Desirée at week 6 (436 DD) whereas fewer were observed on Morag at 14.3 °C at week 6. At 17.3 °C, males were observed again at weeks 11, 13, 14, 15 and 16 with Desirée and at week 15 with Morag. Males were not observed at 14.3 °C for either cultivar after week 11.

Mature (tanned) cysts were recovered from the soil from 9 weeks after inoculation onwards with Desirée at 14.3 °C (655 DD) and 17.3 °C (844 DD), with greater numbers at the higher temperature (Fig. 1e), peaking at 938 DD. Very few cysts were recovered with Morag at either temperature. There was a significant difference in the overall mean number of cysts recorded between the two temperature regimes (14.3 and 17.3 °C), the mean responses at different temperatures ($P = 0.005$) and for different cultivars ($P < 0.001$). There was also an interaction between temperature and cultivar ($P = 0.007$).

Field trials

Soil temperatures

The average soil temperatures at a depth of 20 cm in the potato drills over the growing seasons of 147 and 157 days were 14.1 and 15.5 °C at the East Lothian and Harper Adams sites, respectively (Table 1). The fluctuations in the soil temperature during the growing season at the 2 sites is shown in Fig. 2. The temperatures at Harper Adams were higher than at East Lothian during three of the periods between soil samplings and were particularly high during the final harvest period (average 16.6 compared to 12.9 °C). Assuming a base temperature of 4 °C for *G. pallida* (Ebrahimi *et al.*, 2014), there were 1511 and 1804 DD at the East Lothian site and Harper Adams sites respectively. A *t*-test confirmed significant differences in the soil temperature between the sites ($P < 0.001$).

qPCR validation

The primers and probes for the qPCR assay were designed to be specific to *G. pallida* or *G. rostochiensis* (Reid *et al.*, 2010) and were based on rDNA ITS sequences of many PCN isolates from Europe. All samples were tested for both PCN species but no *G. rostochiensis* was detected. In order to use this assay to quantify *G. pallida* in the field trial soil samples, it was necessary to establish that the relationship between egg numbers and qPCR qty was linear over a wide range of eggs numbers. The DNA yield (qPCR qty) from *G. pallida* cysts was positively correlated with the number of eggs (Fig. 3) giving the linear regression model: $y = 25.367x$ with $R^2 = 0.7619$, where y is the number of eggs of *G. pallida* and x is DNA yield multiplied by 10 for the dilution. Once established, the model was applied to field samples to determine the egg number per sample.

Quantifying PCN in the East Lothian field trial

The initial population (Pi) of the East Lothian field site was an average of 11.7 ± 1.6 (without nematicide plots) and 20.6 ± 2.2 (with nematicide plots) eggs per g soil of *G. pallida*. Changes in the *G. pallida* population over the growing season, without and with nematicide treatment for the four cultivars are presented in Figure 4a and 4b, respectively. In the plots without nematicide treatment, the multiplication ratio (population at sampling:initial population) generally decreased at 4, 8 and 12 weeks, which is consistent with a decrease in the egg content of cysts due to hatching. At week 16, the multiplication ratio peaked. At week 20 (1511 DD) the final Pf⁄Pi (final population⁄initial population) dropped significantly and ranged from 5.0 ± 1.9 for Cara to 2.4 ± 0.4 for Desirée. However, in the nematicide-treated plots, the multiplication remained at similar levels over the whole growing season. The overall trend for the effect of nematicides is shown in Figure 4c, where the multiplication ratio at each sampling time has been averaged over the four cultivars. The final average Pf⁄Pi values were 3.6 ± 0.6 for the untreated and 0.7 ± 0.1 for nematicide-treated plots. The relative increase in the population was clear without nematicide treatment at weeks 16 and 20 in contrast to the nematicide treatment where the final population was lower than the initial population. To assess the influence of nematicide treatment and cultivar on multiplication (Pf⁄Pi), an ANOVA was used. It revealed no significant differences in the reproduction ratio between the cultivars during the growing season; however, there nematicide treatment had a significant influence on population multiplication ($P < 0.001$) over the growing season.

Quantifying PCN in the Harper Adams field trial

The Pi of *G. pallida* in the Harper Adams field trials was estimated prior to planting in April. The average of 9.9 ± 0.6 (without nematicide) and 6.3 ± 0.9 (with nematicide) eggs per g soil was determined by qPCR. Samples were negative when tested for *G. rostochiensis.* Changes in the *G. pallida* population over the growing season in the plots without and with nematicide treatment for the four cultivars are presented in Figure 5a and 5b respectively. For samples

without nematicide treatment, there were generally slight reductions in the population at 4 and 8 weeks after planting compared to preplant levels. At week 16 (1193 DD), the multiplication ratio increased to reach the Pf⁄Pi at final harvest of 7.7 ± 3.0 for Cara and 2.5 ± 0.5 for Estima. For the nematicide treatment, the levels remained similar for the first 12 weeks of the field experiment, but the multiplication ratio increased at the fourth sampling time and at the combined harvests at weeks 20 and 22. The final average Pf⁄Pi values for the four cultivars were 5.2 ± 1.0 and 2.1 ± 0.3 for the untreated and treated plots, respectively (Fig. 5c). To assess the influence of nematicides and different cultivars on multiplication, an ANOVA was performed. There were no significant differences in the Pf⁄Pi between the cultivars during the growing season; however, there was a significant influence of nematicide treatment on Pf⁄Pi ($P < 0.001$) over the growing season.

Relationship between initial population and reproductive factor (Pf⁄Pi)

The Harper Adams site was lightly to moderately infested with *G. pallida* (<20 eggs per g soil) and the site in East Lothian varied from lightly to heavily infested with a range of initial population densities (5–50 eggs per g soil). The results indicated that in the untreated plots at both sites, particularly at East Lothian, there was a trend towards a decreasing multiplication rate with increasing initial population (Fig. 6a). The multiplication rate was significantly higher in the plots with lower initial densities ($P < 0.001$). Also, the multiplication rate (Pf⁄Pi) was reduced with the oxamyl treatment at the East Lothian site. At the Harper Adams trial, no trend was found between Pi and Pf⁄Pi in the nematicide treated plots (Fig. 6b). Further statistical tests (ANOVA) on the multiplication rate revealed a significant effect of initial population on the Pf⁄Pi ($P < 0.001$) and nematicide treatment ($P < 0.001$) at both sites but no difference in multiplication rate associated with different cultivars.

Developmental stages of the nematodes in the roots from the field trials

At the first harvest (week 4; 281 DD) from the East Lothian site, J2 nematodes were most frequently observed in the roots in both nematicide-treated and untreated samples, although single J3 and J4 stages were also found (Fig. 7a, b). At week 8 (593 DD), mainly J3 stage nematodes were observed, and much lower numbers of J2 and a few J4 stage nematodes. There was an increase in the J2 nematodes at week 12 (895 DD), particularly in Desirée roots.

At Harper Adams, J2 nematodes were observed in the roots of both Desirée and Cara at week 4 (278 DD; Fig. 8a, b). The highest number was recorded in Cara from the untreated plots. At week 8 (544 DD), both J3 and J4 stage nematodes were observed. J2 and J3 stages were observed in Cara samples from week 12 (856 DD) in non-nematicide-treated plots, while no nematodes were found in roots from the treated plots. Juvenile stage 2 nematodes were observed to increase in numbers in untreated plots at weeks 20 and 22 (end of experiment 1804 DD).

In the East Lothian root samples, the first occurrence of females and highest numbers were found on roots from Cara and Desirée 8 weeks (593 DD) after planting (Fig. 7c). Lower numbers of females were seen at week 12 (895 DD); however, at week 16 (1189 DD) no females were observed. Surprisingly the highest numbers of females were found with the nematicide treatment. At week 20 the roots had deteriorated, and it was not possible to examine them histologically. A few females were observed on the roots of Cara at week 4 (278 DD) at the Harper Adams site, but most were seen at 8 (544 DD) and 12 weeks (856 DD), with higher numbers observed without nematicide treatment (Fig. 8c). Females were found at combined weeks 20 and 22 (1804 DD) on the roots of Cara and Desirée in the untreated samples, and a few were seen at week 22 (1804 DD) in roots from the nematicide treatment with both cultivars.

Hatching test with field cysts

The cumulative proportion of eggs that hatched from the field cysts from the two sites was calculated for each of the sets of cysts over the 56 days of the experiment (Fig. 9a) and fitted to a linear regression model. Analysis of variance on the log-transformed curve parameters suggested no evidence of differences in the means between the two sites (East Lothian and Harper Adams) for maximum hatch rate or time to 50% hatch. There was a (marginally) significantly higher total proportion hatching at East Lothian than Harper Adams ($P < 0.001$) (Fig. 9b). This is confirmed by ANOVA on the final counts (raw data), where the mean total hatch at East Lothian was 975 and for Harper Adams 711 ($P = 0.03$), and ANOVA on the total proportion hatching. There are also significant differences in the time to 50% hatch and total hatch between temperatures but not for hatching rate.

Discussion

The main goal of these experiments was to investigate the relationship between the life cycle of *G. pallida* and temperature and to assess the potential for the development of a second generation of *G. pallida* in UK fields. The data has also been used for the development of a dynamic stage-structured simulation model (Kettle & Nutter, 2015) and for risk assessments in relation to climate change (Skelsey *et al*., 2018). The growth cabinet experiments provide a temporal framework for the rates of development in different temperature regimes and indicate the relative abundance of the different life stages in different temperature regimes. The pot experiment was performed to allow comparison between the life cycle of *G. pallida* in a controlled environment and in more variable field conditions. There was no effect of the

potato cultivar on the number of juveniles recovered from the soil in the initial hatching peak. This is in agreement with Turner (1990) who found no significant difference in hatching of *G. pallida* with various *S. vernei* hybrids. In previous *in vitro* hatching experiments conducted in petri dishes using potato root diffusate to induce hatching(Kaczmarek *et al.*, 2014a, b)) , newly hatched juveniles of *G. pallida* were first observed at day 9; however, more hatched by day 12 at 13–25 °C. The results obtained with the growth cabinet experiments using potato plants in soil, showed a similar delay before hatching occurred observed by the presence of juvenile nematodes recovered from the soil, and hatching continued for several weeks. Adult males were recovered from the soil at 333 DD and the maximum number of males was recorded about 430 DD in both temperature regimes; however, more males were recorded for the growth cabinet with the lower temperature. Further investigation is needed to determine if there is an effect of temperature on sex determination (Jones *et al.*, 2017; Skelsey *et al.*, 2018).

In the present investigation, new cysts were observed in the 17.3 °C regime from nine weeks after inoculation (844 DD), followed by the start of a second increase in the number of juveniles in the soil at week 10 (938 DD), indicating that fewer than 10 weeks were required for one generation to be completed and for another to start in these conditions for this population of *G. pallida*. The juveniles observed at 10 weeks (938 DD) and later are consistent with the hatching of a second generation originated from new, first generation females; this implies that diapause was not obligatory for all juveniles from the first generation, although the amount and timing of the second hatch was affected by the temperature regime. Hatching with PCN is not tightly synchronized, resulting in the occurrence and overlapping of different developmental stages over several weeks. Nevertheless, based on these results, if the second generation develops at the same rate as the first generation at this temperature, then second generation cysts would be expected at week

18 (about 8 weeks after the second appearance of juveniles in the soil). Because it was not possible to sustain the plants for longer than 16 weeks in the growth cabinets despite regular fertilization and watering, it was not possible to determine whether a second increase in cysts would occur.

In both temperature regimes, lower numbers of cysts were observed with partially resistant Morag compared to Desirée, consistent with previous reports (Phillips *et al.*, 1980; Phillips & Trudgill, 1998). However, there was a much greater response to temperature with susceptible Desirée. This indicates that partially resistant cultivars may be more effective than susceptible cultivars in controlling population multiplication over a wider temperature range. Significantly lower egg numbers per cyst were found with Morag than with Desirée, but numbers did not differ significantly between the two temperature regimes. This contrasts with other reports (Bendezu *et al.*, 1998; Da Cuhna *et al.*, 2012) where resistance from *S. vernei* did not significantly reduce the number of cysts or egg content in more virulent populations of *G. pallida* from Portugal and Bolivia, although with this source of resistance, less virulent populations of *G. pallida* had significant differences in their multiplication.

The experiments in temperature-controlled environments described here used a PCN population from the JHI collection that has been multiplied in glasshouse conditions and may have become adapted to these conditions. Other work has noted differences between populations of *G. pallida* in their temperature responses (Foot, 1978) and a population of *G. rostochiensis* was selected that matured more quickly at low temperatures following several years of early cropping in Ayrshire, UK (Ellenby & Smith, 1975). However, the hatching tests over a range of temperatures performed in the present study with *G. pallida* cysts isolated from two field sites confirmed the responses obtained previously with glasshouse cultured *G. pallida* (Kaczmarek *et al.*, 2014 a, b).

The qPCR assay for *G. pallida* and *G. rostochiensis* (Reid *et al.*, 2010) was used to monitor the densities of eggs per g soil in soil samples taken at the two field sites. This is the first time this assay has been used to monitor PCN population dynamics in the field over a growing season and demonstrates the value of this high-throughput assay for assessing the impact of different PCN control programmes. The relationship between egg numbers and DNA yield was validated using *G. pallida* cysts recovered and quantified from soil using the same extraction procedure used for the field samples.

The mean soil temperature between planting and first harvest was 13.9 °C at the Scottish site compared to 14.4 °C at the English site, below the optimal temperatures that have been observed for hatching (Kaczmarek *et al.*, 2014a, b). At harvest 1 at East Lothian but not in the Harper Adams root samples, J3 stage nematodes were observed and a few female nematodes were observed on the roots from Harper Adams. The mean soil temperatures in sampling periods 1 and 2 were lower at Harper Adams than the East Lothian site; however, they increased to 15.5 and 16.6 °C in sampling periods 4 and 5 giving 1193 and 1804 DD respectively, whereas they dropped to 14.4 and 14.1 °C at the East Lothian site with 1189 and 1511 DD respectively. Females were observed on the roots from week 4 onwards at Harper Adams with relatively warmer soil temperatures (14.8–15.5 °C) as found by Jones *et al.* (2017). It is probable that a second generation developed, as both juveniles and females were observed at harvests 5 (week 20) and 6 (22 weeks), although the possibility that the late females observed in the field samples may have resulted from a delayed initial hatch cannot be discounted. However, two generations of *G. pallida* in a growing season has been reported with high numbers of cysts in soil temperatures from 18 to 22 °C in Avezzano, Italy (Greco *et al.*, 1988) and in the present study, a second hatch was observed at 1219 DD in the controlled environment cabinet at 17.3 °C. Ebrahimi *et al.* (2014) estimated the degree day requirement for *G. pallida* of 450 DD with a 4 °C base temperature. There were 1511 DD

449 and 1842 DD at the East Lothian and Harper Adams sites respectively, both sufficient for two
450 generations to be completed.

451 The general trend of increasing temperatures associated with climate change
452 combined with the warmer conditions that occur in some regions in the UK are making it
453 more likely that conditions that support two generations of *G. pallida* within a growing
454 season will occur. A recent report showed that both species of PCN are developing more
455 quickly in Belgium than in the past (Ebrahimi *et al*., 2014) and this has resulted in a change
456 of advice for the time of harvesting early cultivars to prevent PCN from completing its life
457 cycle. In 2014 in Lincolnshire, average soil temperatures at a depth of 20 cm of 15.3 °C were
458 recorded and many females were observed developing on roots of susceptible untreated
459 potatoes 21 weeks after planting (A. Barker, Barworth Agriculture LTD, Sleaford, personal
460 communication). It is not known whether these late developing females matured into cysts or
461 whether they contained viable eggs that could contribute to a higher final Pf/Pi; this requires
462 further investigation. In some conditions, a partial second generation, where a substantial
463 number of nematodes hatch but are not able to complete the second generation of mature
464 cysts, could lead to lower final Pf/Pi values and a trap crop effect. An effect of temperature on
465 in-egg mortality and spontaneous hatching, affecting population dynamics, has also been
466 reported (Devine *et al.*, 1999).

467 Nematicides can affect PCN during different developmental stages, mainly affecting
468 juvenile nematodes before they reach the potato plants. Fosthiazate temporarily inhibited
469 hatching of *G. pallida* in an *in vitro* test and suppressed hatching in soil (Woods *et al.*, 1999).
470 The results of the nematicide trial in the current investigation are consistent with other studies

471 that have reported that carbamate nematicides do not permanently stop hatching in
472 field conditions (Evans & Wright, 1982; Woods *et al.*, 1999); nevertheless, they do delay the

process significantly. Although the decline in egg numbers was less in nematicide treated plots than in untreated plots, this was probably due to less hatching with the nematicide treatment. Generally, fewer J2 stage nematodes were observed in the roots of Cara and Desirée with nematicide treatment, consistent with a report by Minnis *et al.* (2004) and fewer females were observed at week 8 with the nematicide treatments at both sites. At the East Lothian site there was significantly lower multiplication with the oxamyl treatment ($P < 0.001$). This is in contrast to the results of Minnis *et al.* (2004) that showed no significant difference in PCN multiplication between fields treated or untreated with oxamyl. However, as mentioned previously, in the current investigation, the density of the initial population in untreated plots ranged from 5 to 20 eggs per g soil while the plots for nematicide treatment had >20 eggs per g of soil; therefore, it is difficult to differentiate between a reduction in the multiplication rate due to the nematicide treatment or due to the higher initial number of eggs in the soil.

Understanding how climate change may drive the life cycle and population dynamics of PCN is important because of the role of these plant pests in potato yield reduction; in addition, the management of this pest is of increasing concern due to the withdrawal of nematicides from use. The results indicate that the development of multiple generations of PCN within one growing season in the UK is more likely with increasing soil temperatures associated with climate change and this could have significant effects on management of population multiplication and prevention of the spread of PCN (Jones *et al.*, 2017; Skelsey *et al*., 2018).

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595

596 **Figure legends**

597 **Figure 1** Comparison of number *of Globodera pallida* juveniles at 14.3 °C (a) and 17.3 °C
598 (b), males at 14.3 °C (c) and 17.3 °C (d), and cysts at 14.3 and 17.3 °C (e) recovered from the
599 soil over 16 weeks in the growth cabinet experiment with susceptible cv. Desirée and
600 partially resistant cv. Morag. The number of degree days (DD) at each sampling time are
601 given in brackets. Two pots were used for each temperature and cultivar combination, and
602 nematodes were extracted with a Baermann funnel. The bars indicate the standard error of
603 means.

Figure 2 Soil temperatures at 20 cm in potato drills over the growing season in Harper Adams (4 April 2011 to 6 September 2011, 155 days) and East Lothian (3 May 2011 to 28 September 2011, 147 days). Soil temperatures were taken every 3 h in two adjacent drills and the averages are shown.

Figure 3 Linear regression between egg numbers of *Globodera pallida* and quantity of copies determined by qPCR. The qPCR results for 11 samples were averaged across three qPCR replicates.

Figure 4 Changes in the multiplication ratio (population at sampling time/initial population) of *Globodera pallida* over the growing season (a) without nematicide treatment, (b) with nematicide treatment, and (c) average multiplication ratio for the four cultivars (Cara, Desirée, Estima and Maris Piper) with and without nematicide treatments at the East Lothian site. Soil samples were taken at planting and at 4-weekly intervals (degree days (DD) are shown in brackets after each sampling time) and the eggs per g soil determined from isolated cysts using qPCR. The bars are standard errors of the means for each cultivar.

Figure 5 Changes in the multiplication ratio (population at sampling time/initial population) of *Globodera pallida* over the growing season (a) without nematicide treatment, (b) with nematicide treatment and (c) average final population/initial population (Pf/Pi) for the four cultivars (Cara, Desirée, Estima and Maris Piper) with and without nematicide treatments at the Harper Adams site. Soil samples were taken at planting and at 4-weekly intervals (degree days (DD) are shown in brackets after each sampling time) and the eggs per g soil determined from isolated cysts using qPCR. The bars are standard errors of the means for each cultivar.

Figure 6 Reproductive factor (ratio of final population/initial population (Pf/Pi)) versus initial population (Pi) of *Globodera pallida* at **(**a) East Lothian and **(**b) Harper Adams sites.

Figure 7 The numbers of J2, J3, J4 and females of *Globodera pallida* observed inside 100 g of acid fuchsin-stained roots of cultivars Cara and Desirée from the 2011 field trials at East Lothian. (a) Non nematicide-treated plots, (b) nematicide-treated plots, and (c) females from both treatments. Root samples were examined at harvests 1, 2, 3 and 4 (weeks 4, 8, 12 and 16, respectively, which correspond to 281, 593, 895 and 1189 degree days (DD)). The bars indicate the standard error of the mean.

Figure 8 The numbers of J2, J3, J4 and females of *Globodera pallida* observed inside 100 g of acid fuchsin-stained roots of cultivars Cara and Desirée from 2011 field trials at Harper Adams; (a) non-nematicide-treated plots, (b) nematicide-treated plots, (c) females from both treatments. Root samples were examined at harvests 1, 2, 3, 4, 5 and 6 (weeks 4, 8, 12, 16, 20 and 22, respectively, which correspond to 278, 544, 856, 1193 and 1804 degree days). The bars indicate the standard error of the mean.

Figure 9 Proportion of hatched eggs of *Globodera pallida* from the field populations in potato root diffusate, during 55 days incubation at different temperatures; (a) cumulative and (b) percentage of total hatch. Bars indicate standard errors of means.