R433 Population dynamics of potato cyst nematodes in relation to temperature

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The summary below was produced by Dr VC Blok

Summary

- Multiplication of potato cyst nematodes is temperature dependent. Increases in soil temperatures above the current range that were recorded in potato drills in Scotland and England will favour both faster and greater hatching and multiplication. In general, the soil temperatures tended to decrease with increased latitude and thus higher multiplication is likely to occur in southern UK although there was considerable variation between sites within the season and between seasons.
- The rate and amount of hatching is temperature dependent. Soil temperatures following planting are predicted to have a significant effect on the amount of hatching and the potential for population multiplication. The population of *G. pallida* tested hatched more at lower temperatures than the *G. rostochiensis* populations and *G. rostochiensis* hatched more at higher temperatures than *G. pallida*. A model has been developed describing the relationship between temperature and hatching for both species.
- The occurrence of a second peak of juvenile nematodes was observed in pot experiments and is consistent with the start of a second generation. This implies that entry into dormancy at the end of the first generation is not obligatory. The amount of juveniles in the second peak increased with increasing temperatures. The start of the second peak occurred earlier with *G. rostochiensis* (9 and 10 weeks at 18º and 14ºC respectively) than *G. pallida*

(10 and 11 weeks at 17º and 14ºC respectively) consistent with the former having a faster life cycle at favourable temperatures.

- At 17ºC one generation of *G. pallida* required 63 days. An average soil temperature of 17º was recorded at Luffness in 2010 and several other sites had average soil temperatures of >16º. This implies that 2 generations could be possible in ~18 weeks and given the faster life cycle of *G. rostochiensis*, 2 generations would take less time with this species.
- No difference was observed in the hatching response to susceptible Desirée or the partially resistant Morag with *G. pallida.* Fewer cysts and fewer eggs/cyst developed on the partially resistant Morag compared to the susceptible Desirée and cysts took longer to develop on Morag.
- A dynamic stage-structured simulation model has been developed by Helen Kettle (BioSS) using the PCN temperature data which allows the number of nematodes in each life stage to be evaluated at any moment in time, based on a time-series of soil temperatures. Given the correct parameter values, the number of generations of PCN that can survive and the number of eggs remaining at the end of the growing season for different soil temperatures can be predicted.
- In field trials at Harper Adams, England and Luffness, Scotland, the *G. pallida* populations' were monitored monthly. At Harper Adams, which had an average soil temperature of 14.85ºC there was evidence of a second generation based on the presence of juveniles and females in the roots in the latter part of the growing season. Luffness had an average soil temperature of 14.15°C and was cooler in the latter part of the growing season and there was little evidence of a second generation. There was a decrease in eggs/gm soil at both sites with nematicide treatments.
- Examination of PCN population dynamics using the automated cyst extraction system based at SASA combined with DNA extraction and qPCR was demonstrated for the first time.
- Cysts of *G. pallida* from the Harper Adams and Luffness sites were characterised as mixtures of 2 introductions from South America based on cytochrome B sequencing. The Pa1 pathotype was found in a farm in East Lothian.

Recommendations for future development of PCN model(s):

- Temperature is an important factor in PCN population dynamics. The occurrence of 2 generations of PCN on susceptible cultivars of potato, in some UK locations that currently have the warmest soil temperatures is predicted and further work to monitor population multiplication during the growing season in the areas most at risk (i.e. East Anglia) should be undertaken. Management options to minimise the risk of 2 generations should be investigated.
- Additional monitoring of soil temperatures in the UK is recommended to better understand regional differences and within and between seasonal variations.
- A comparison of PCN field populations is recommended to assess whether there are regional adaptations to temperature differences.
- The importance of partial hatch in relation to decline rates should be considered.

Introduction

The potato cyst nematodes (PCN) *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone) are economically important pests of potato and are difficult to control because of their long persistence in soil. Both PCN species are listed as EPPO A2 Annex 1/A2 quarantine pests and are found in most EU Member States. The latest European Council Directive concerning PCN (2007/33/EC) came into effect on July 1, 2010 and introduced further measures to suppress and prevent the spread of PCN in member states and requires an annual survey of ware land and implementation of a control program where PCN is found. The withdrawal of nematicides for the control of PCN by the EU due to concerns over their persistence in the soil and non-target effects and the limited availability of cultivars with resistance to *G. pallida* highlight the pressing need for better methods to manage PCN. The Potato Council/SCRI model for PCN was originally developed as an education tool to explain how different factors affect yield and final population levels of PCN. This work has been funded to understand if there are aspects of the model which need further development and/or modification.

Scope

In the UK there has been a shift in prevalence from *G. rostochiensis* to *G. pallida* which is attributed to the growing of potato cultivars that are resistant to the former. However, both species are frequently found in the same field and a large proportion of the potatoes cultivated are susceptible to both species. This project aims to investigate how the two species compete on different hosts which are susceptible or resistant to one or both of the species. Preliminary results suggest that there may be additive benefits when different sources of resistance are combined which may be useful in situations where mixtures of the two species occur. These results also suggest that combining different sources of resistance may have benefits for controlling a single species. The mechanisms underpinning additive benefits of combining resistances warrant further investigation to determine the practical utility of this.

There are reports in the literature that the temperature responses of the two species of PCN differ, however the implications for inter-specific competition between the two species have not been investigated. In temperate countries it has been generally accepted that PCN undergoes a single generation during the potato crop followed by an obligate dormant stage (diapause) which is not broken until the nematode has experienced a winter. However, partial and complete second generations have also been reported and this has significant implications for the control of PCN. If the second generation is completed this could lead to large increases in population sizes as well as applying strong selection for genotypes with faster life cycles and/or increased virulence. Alternatively, a partially completed second generation might also lead to lower final population levels if the egg content of the first generation is significantly reduced while the second generation is not completed at harvest. The implications for damage to the host by a second generation nor the possibility that cysts in which a significant number of eggs have hatched may be more vulnerable to degradation have not been investigated. In addition, the current Potato Council/SCRI model has not considered the implications of a second generation and may require revision.

Methods, key results and conclusions

1) Determine how mixtures of the two species of PCN compete on different genotypes of potato in which resistance or susceptibility to one or other species are combined

A pot experiment was conducted in the glasshouse in 2011 to examine competition between *G. pallida* E/Lindley and *G. rostochiensis* A (Ro1) on the susceptible cultivars Desirée and Maris Piper, and on Vales Everest which has partial resistance to *G. pallida* derived from *S. tuberosum* spp. *andigena* (*H3*). Tubers were planted into a 1:1 mix of sterile sand:loam and after one week were inoculated with eggs comprised of different mixtures of *G. pallida* and *G. rostochiensis* or one or the other species separately. Ten weeks after inoculation, watering of plants was stopped, the soil was dried and cysts were extracted at SASA, then cleaned further by acetone floatation and counted.

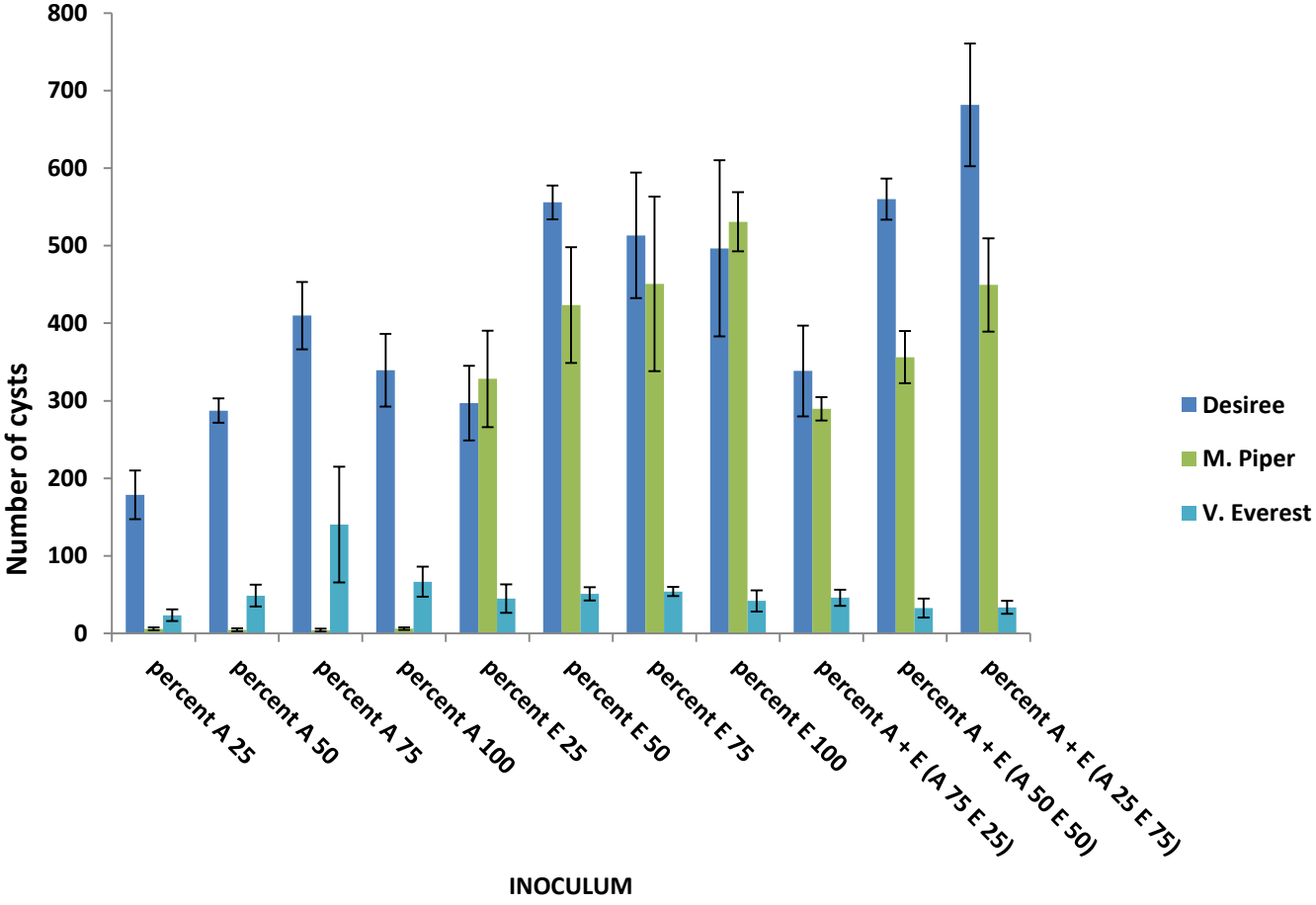


Figure 1 Number of cysts produced by *G. rostochiensis*, *G. pallida* or mixtures of the 2 species with different amounts of inoculum (25, 50, 75 or 100% and mixtures to obtain the equivalent number of eggs as 100% of either species individually) and on 3 potato cultivars, Desirée, Maris Piper and Vales Everest. Bars are standard errors.

Multiplication of both *G. pallida* and *G. rostochiensis* separately on Desirée showed a general trend of increasing multiplication with increasing amount of inoculum. Multiplication of *G. rostochiensis* on Maris Piper was minimal whereas *G. pallida* multiplication was similar to that on Desirée. Multiplication of *G. rostochiensis* on

Vales Everest was higher than on Maris Piper but much reduced compared to Desirée demonstrating partial resistance to this species in Vales Everest. Multiplication of *G. pallida* on Vales Everest was greatly reduced compared to Desirée and Maris Piper but was higher than multiplication *of G. rostochiensis* on Maris Piper. When the 2 species were combined there was an increasing amount of multiplication on both Desirée and Maris Piper as the proportion of *G. pallida* in the mixture increased which is consistent with the generally higher multiplication seen with this species even when similar amounts of inoculum was used for both species. Multiplication of mixtures on both species on Vales Everest was low and did not show an increase in susceptibility when both species were present indicating that the resistance in this cultivar is at least as effective when both species are present as when they are present as single species.

A second competition experiment was performed in 2012 to compare competition by *G. pallida* and *G. rostochiensis* on Desirée with the recently produced cultivar Mistay (Gladiator (*H1*, 62.33.3) x Innovator (*S. vernei*)) and the advanced breeding line 05.Z.39 A 35 (Vales Everest (*H3*) x Valor (*H1*)). This experiment was performed in the glasshouse and then replicated in an unheated polytunnel to examine competition between the species and on 3 different potato genotypes and in 2 different environments. At the end of the experiment cysts were extracted at SASA, acetone floated and then counted.

The plants in the glasshouse experiment succumbed to a fungal infection and growth was poor. Generally multiplication was poor and highly variable compared to the experiment in 2011 with cysts numbers generally 1/10 less than in 2011. There was multiplication on Mistay and 05.Z.39 A 35 by both species but it is not possible to assess the relative multiplication with this experiment. The survival of plants in the polytunnel was vpoor, and while cysts were recovered from most pots, the numbers were low and highly variable.

To determine the proportion of the 2 PCN species in the cysts obtained with mixed inoculum in the 2011 experiment, DNA was extracted from the cysts and a PCN qPCR assay was performed at SASA. This required validation of the qPCR assay with a wide range of cyst numbers (Figure 2) and egg numbers (results not shown) to show that the DNA yield was correlated with the number of cysts and eggs. For technical reasons the application of the qPCR to the experimental samples requires repeating.

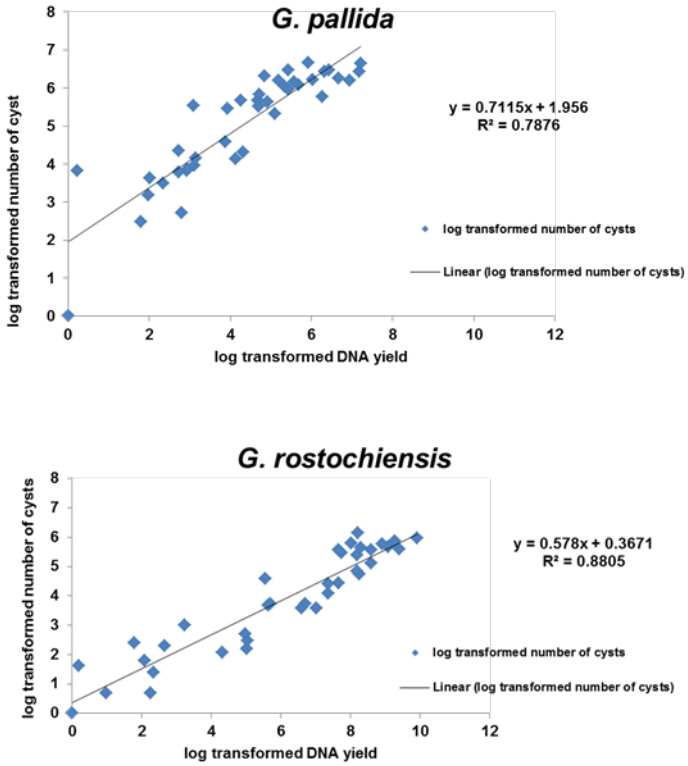


Figure 2 Correlation between DNA yield and numbers of cysts of *G. pallida* and *G. rostochiensis*.

2) Investigate the mechanisms in which combining resistances provide additional control of PCN

The competition experiment performed in 2011 clearly demonstrated that resistance can be combined in one cultivar (ie Vales Everest) that greatly reduces multiplication of both species of PCN and that it is as effective when the species are mixed or separate. In contrast, the single major resistance *H1* is specific to *G. rostochiensis* and does not affect multiplication of *G. pallida* even in the presence of *G. rostochiensis*. The competition experiment in 2012 was aiming to investigate the multiplication of mixtures of both species with potato genotypes with different combinations of resistance and then to investigate synergistic effects if they occurred. However, as the 2012 experiments failed it was not possible to do this.

3) Calibrate the relationship between temperature and life cycle of both species of PCN

The aim of this section was to obtain quantitative data concerning the relationship between temperature and the multiplication of both species of PCN in order to develop a model to use in predicting multiplication of PCN at different temperatures and of the risk of 2 generations of PCN occurring within one growing season currently, and in the future, in the UK.

i) Hatching of G. pallida *and* G. rostochiensis *over a temperature gradient with constant temperatures.*

The initial part of the PCN life cycle, hatching, was examined in relationship to temperature. The rates and total amounts of hatching were determined. 10 selected cysts of either *G. pallida* and *G. rostochiensis* were placed in 5cm petri dishes to which 5ml of potato root diffusate (PRD) or H_2O (5 or 2 replicates respectively) was added. The dishes were arranged in rows on a temperature gradient table with temperatures ranging from 5-29ºC. Numbers of hatched juveniles was determined twice/week for 35 days and the final eggs remaining in each dish were determined so that the proportion of hatching could be calculated.

Hatching by both species was significantly temperature dependent. *G. rostochiensis* hatched more readily than *G. pallida* in PRD at the higher temperatures whereas *G. pallida* was able to hatch more than *G. rostochiensis* at the lower temperatures (Figure 3). Overall *G. pallida* had a higher percentage hatch although this might be due to the particular population that was used. *G. rostochiensis* showed an increasing amount of hatching as the temperatures increased from 11-21ºC whereas for *G. pallida* the amount of hatching was similar between 13-23ºC (Figure 3). *G. rostochiensis* hatched more quickly than *G. pallida* and both species showed a delayed second hatch particularly at the higher temperatures (Figure 4). There was little hatching with either species in H_2O or below 7º.

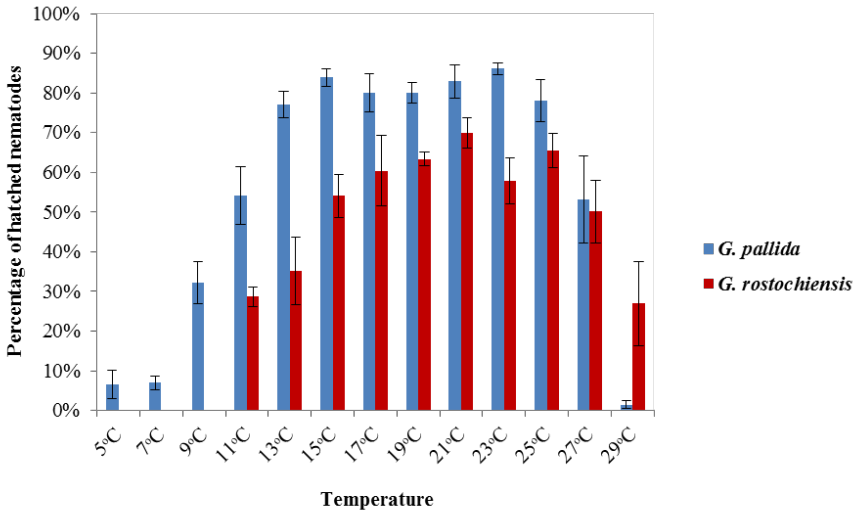


Figure 3 Effect of different constant temperatures ranging from 5 – 29ºC on hatching of *Globodera rostochiensis* and *G. pallida* in potato root diffusate (PRD). Data are expressed as a percentage of total nematodes that hatched over 35 days of the incubation at each temperature and are means of 5 replicates. The bars indicate the standard error of the means.

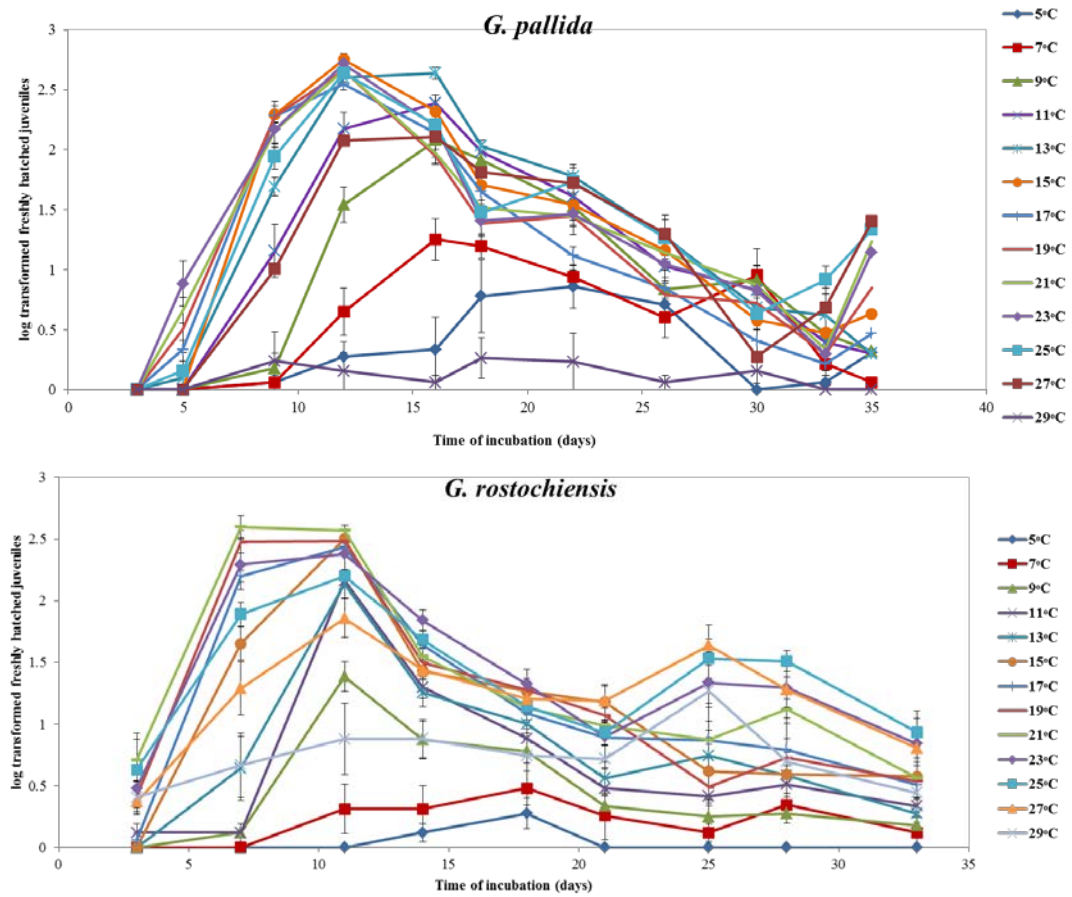


Figure 4 Numbers of freshly hatched juveniles of *G. rostochiensis* and *G. pallida* and in PRD over 35 days of incubation over a temperature gradient from 5-29ºC. Vertical lines indicate standard error of the means of the 5 replicates.

Cysts used for this study were taken from one population of each species that had been multiplied and stored under well-defined conditions. The main goal of the hatching experiments was to investigate the differences in the hatching reactions of the *G. pallida* and *G. rostochiensis* in different temperature regimes and to determine if either species might have an advantage in particular soil temperature profiles. Establishing the total hatch in PRD and expressing the number of hatched juveniles in the different temperature conditions as a percentage of total eggs allowed a comparison between both species. The two species were found to differ in their responses to temperature as previously reported. For example, *G. rostochiensis* generally hatched more quickly than *G. pallida*.

The partial hatching that was observed at non-optimal temperatures has implications for the proportion of viable eggs that remain in the cyst which could hatch later, either during the same crop, or in the future. It is possible that these differences in the proportions of unhatched eggs could affect population decline rates. The role of temperature in decline rates merits further investigation.

ii) Hatching of G. pallida *and* G. rostochiensis *over a temperature gradient with fluctuating and constant temperatures.*

In field conditions soil temperature fluctuates during the day and between days. To determine if hatching differed between constant and fluctuating temperatures, a hatching test was performed with the gradient table which had both positions with constant and average temperatures. In order to assess whether a diurnal temperature fluctuation influenced the hatching of PCN, a temperature gradient table

was programmed to produce positions on the table with temperatures that changed every 12 hours and positions with constant temperatures (Figure 5). The average temperatures ranged from 8º to 17ºC.

12	13	14	15	16	16	17
12	13	13	14	15	15	16
11	12	13	14	14	15	16
11	11	12	13	13	14	15
10	11	11	12	13	13	14
9	10	11	11	12	11	13
8	9	9	10	10	11	12

Figure 5 Average temperatures at the 49 positions on the temperature gradient table at the positions where the Petri dishes were placed for the hatching test. The positions (green) on the diagonal from bottom left to top right remained constant, while the others changed every 12 hours with a maximum change from 8º to 17ºC at the top left and bottom right corners.

Both species had higher hatching rates at the warmer temperatures. The percentage of total hatched nematodes for both PCN species at the average daily temperatures in the gradient is shown in a Figure 6. Due to the design of the gradient table, some temperature combinations had only one sample therefore estimating the variability for these was not possible.

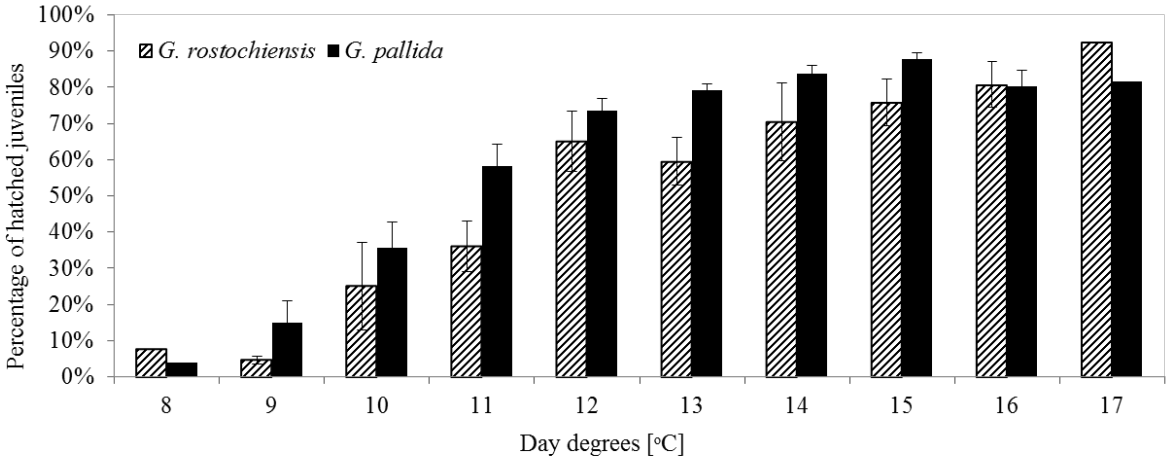


Figure 6 Total hatching of *G. rostochiensis* and *G. pallida* in potato root diffusate (PRD) after 33 days over a temperature gradient from 8º –17ºC with constant or diurnal temperature fluctuations. Data are expressed as percentages of the total hatch per mean daily temperature (day degrees).

The total percentage hatching of PCN over a range of temperatures was shown to have a hyperbolic shape and that the parameters A, B, C and M of the logistic curve Y=A + C/(1 + EXP(-B*(t - M))) vary according to the mean temperature. Boxplots of the curve parameters are shown in Figure 7. Group 1 comprised the hatching data from constant temperatures and Group 2 comprised the data at positions where the temperature was fluctuating (diurnal). Figure 7 shows the curve parameters for *G. pallida* and *G. rostochiensis* split according to group. For both species, the figure

shows that there are no differences in the medians for Group 1 and 2 (the constant and diurnal temperature regimes) for A, B, C and M, though there are differences in the variability and differences between the species.

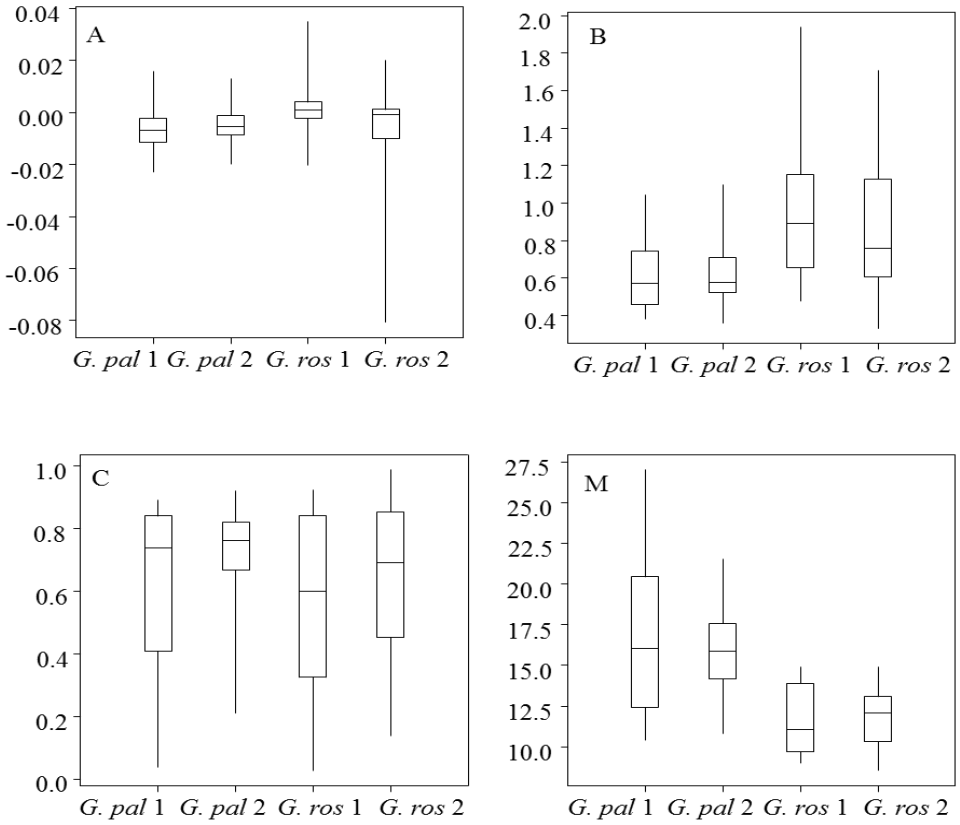


Figure 7 Boxplots of parameters A, B, C, M for the logistic curves fitted to the cumulative proportion of total hatch for *G. pallida* (*G. pal*) and *G. rostochiensis* (*G. ros*) grouped by (1) constant temperature regime or (2) fluctuating temperature regime.

The ANOVA test comparing the responses over the combined diurnal and constant temperature regimes for *G. pallida* and *G. rostochiensis* for parameter M, which concerns the number of days until half of eggs that are going to hatch, have hatched, indicates that *G. rostochiensis* hatched earlier than *G. pallida* ($P<0.001$). The ANOVA for parameter B indicates that *G. rostochiensis* hatched at a faster rate than *G. pallida* ($P<0.001$). Parameter C showed a higher proportion of *G. pallida* eggs hatched overall than *G. rostochiensis.* The ANOVA for parameter C, confirmed that there are significant differences between the species in the means of the final proportion of hatched nematodes ($P=0.005$) and at different temperatures ($P<0.001$) however there was no significant difference in the overall PCN response to temperature ($P=0.218$).

The main goal of these experiments was to investigate whether there were differences in the hatching reaction of PCN in different temperature regimes. In the field, fluctuations in temperature occur. We wanted to determine if hatching rates at constant temperatures were equivalent to hatching rates at comparable average temperatures that were achieved with fluctuating temperatures. We found that hatching rates in constant and fluctuating temperatures were not significantly different for both species of PCN, though the species did differ in their hatching responses to temperature. These studies were performed with populations that have been cultured in the glasshouse for many generations and need to be confirmed with populations from the field. Low soil temperatures typically found shortly after potato

planting in the UK are likely to favour *G. pallida* whereas warmer temperatures are likely to favour *G. rostochiensis*. This has implications for interspecific competition between the 2 species at different temperatures when they occur as mixtures in the field, the host response to mixed infections and the composition of the final PCN populations. Our data also indicate that the hatching response is greater and faster at the higher temperatures tested and thus increases in soil temperatures due to regional climatic differences or climate change are likely to favour PCN multiplication.

iii) Development of G. pallida *and* G. rostochiensis *in different temperature regimes in controlled environments.*

The life cycles of *G. pallida* and *G. rostochiensis* were compared in different temperatures and with different potato genotypes with pot experiments conducted in growth cabinets which had controlled environments and in canisters over a temperature gradient.

For the pot experiments, the occurrence of juveniles and males in the soil (2 reps) with Desirée or Morag (11305) was monitored weekly by extracting nematodes from the soil. The number of new cysts in the soil and the egg content of the cysts were also determined (section 5). Results are shown for *G. pallida* (Figures 8 and 9) and comparable results were obtained with *G. rostochiensis* (results not shown). The growth cabinets had average temperatures of 14 and 17º with day (16h) and night (8h) temperatures that differed by 5ºC. The initial inoculum of 30 cysts was placed in a bag that was removed after 5 weeks.

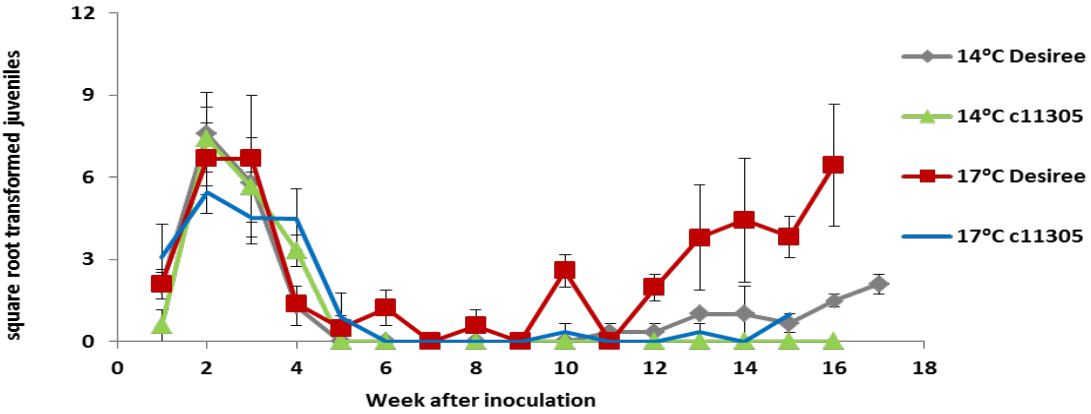


Figure 8 Square root transformed number of juveniles of *G. pallida* in soil over 17 weeks of incubation in growth cabinet experiment at 14°C and 17°C temperatures with cvs Desirée and Morag. The bars indicate the standard error of means.

The highest numbers of juveniles were recovered from the soil in the first peak between weeks 2-4 for both cultivars. Both cultivars (Desirée and Morag) produced similar numbers of juveniles in both temperature regimes. With cv Desirée a second peak of juveniles was observed after 10 weeks at 17º (perhaps a delayed initial hatch) with more juveniles observed after 11 weeks. A smaller and later second hatch was observed at 14º with Desirée (Figure 8). An increase in juveniles was also observed with cv Morag at 15 weeks at 17ºC but a second hatch was not observed at 14º. If time required between the start of the first and second peaks of juveniles was either 9 or 11 weeks (depending if the juveniles at week 10 were from a second hatch or a delayed first hatch) at 17ºC for Desirée ANOVA performed on the number

of juveniles with cultivar, temperature and time as a factors showed a significant influence of temperature on hatching and a significant difference in the number of juveniles that occurred on cvs Desirée and Morag.

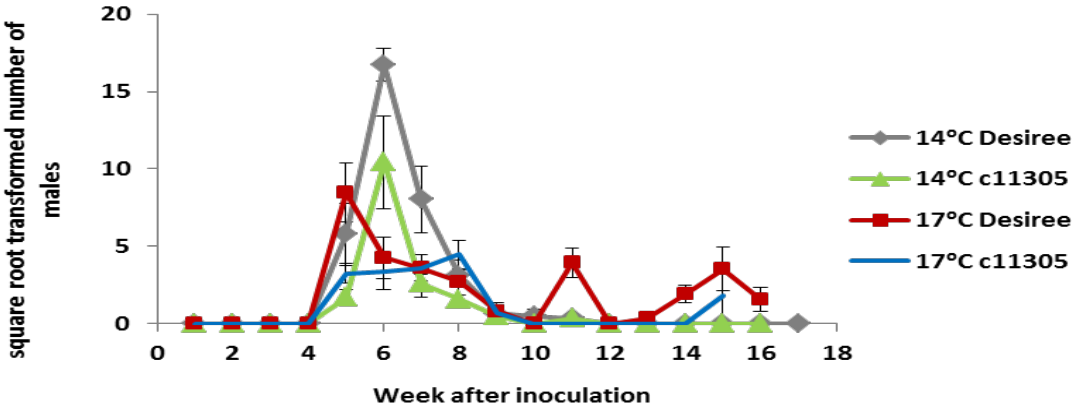


Figure 9 Square root transformation of the number of males *of G. pallida* recovered from the soil over 16 weeks in the growth cabinet experiment at 14°C and 17°C. The bars indicate the standard error of means.

The highest numbers of males were found at 14°C on the cv Desirée at week 6 although males were observed for both cultivars and in both temperature regimes at week 5 (Figure 9). The males observed at week 11 with Desirée at 17ºC may have been from the delayed first hatch. Males appeared again at 14 and 15 weeks with cv Desirée and Morag respectively at 17ºC. Statistical tests on *G. pallida* males revealed no significant effects of the temperature with cv Desirée or Morag on male development (P=0.729) over the time course. Significantly lower numbers of males were observed with cv Morag (P<0.001). With the pot experiment with *G. rostochiensis,* juveniles were observed 2 weeks after inoculation at 18ºC and the second peak started at week 9 with Desirée, and males were observed at week 5.

With the canister experiment, the appearance of females was monitored weekly following the inoculation with eggs. The results for G. *pallida* are shown in Figures 10 and 11 for Desirée and Vales Everest respectively (comparable results for *G. rostochiensis* with Desirée and Maris Piper are not shown).

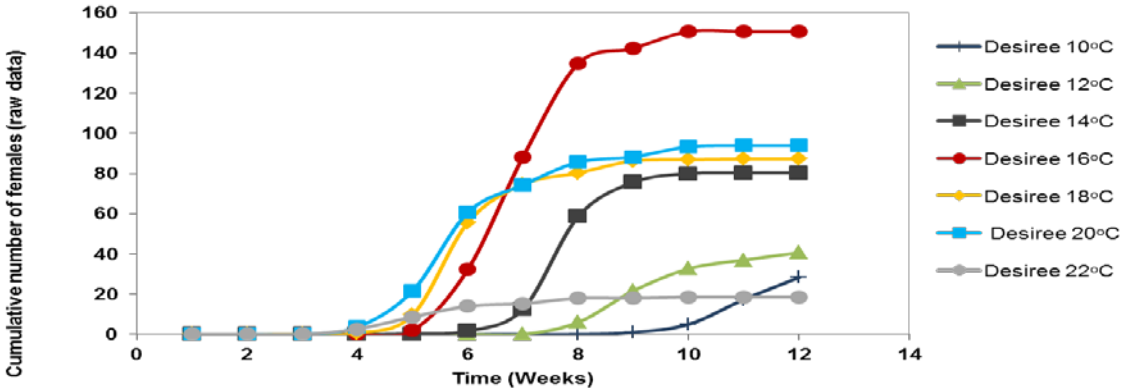


Figure 10 Cumulative number of females of *G. pallida* observed over 12 weeks at different temperatures (10-22°C) on the cv Desirée.

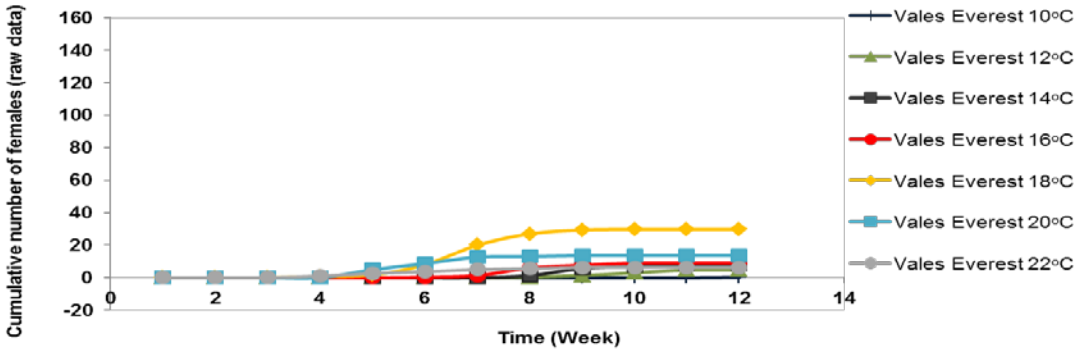


Figure 11 Cumulative number of females of *G. pallida* observed over 12 weeks at different temperatures (10-22°C) on the cv Vales Everest.

The differential effect of temperature on the development of PCN females is shown in Figure 10 and 11. With Desirée, while the highest overall number of females was observed at 16ºC, the females first appeared on the roots at 18, 20 and 22ºC four weeks after inoculation. Most females emerged between weeks 5-9 at temperatures between 14 - 22°C; however, the number of females observed was highest at 16ºC and lowest at 22ºC within this temperature range. The total number of females observed was similar for 20, 18 and 14ºC though it took longer for females to emerge at the latter temperature. The latest first occurrence was recorded at 10°C and 12ºC, at 9 and 8 weeks, respectively, with a few more females observed at these temperatures each subsequent week until the end of the experiment at 12 weeks. The fewest overall numbers of females were observed at 10, 12 and 22ºC (Figure 10).

The numbers of females on roots of cv Vales Everest were significantly reduced compared to cv Desirée ($P<0.001$). As with cv Desirée, the first females appeared at 18, 20 and 22°C and the maximum number of females was observed between 5 and 9 weeks after inoculation. Moreover at 10°C no females were observed on cv Vales Everest (Figure 4). Temperature had a strong effect on the number and rate of appearance of the females.

The days required for females of *G. pallida* to occur on the roots of Desirée at different temperatures is described by the equation 25.9 + ((T-21.9)/1.85)^2 where T is temperature (Figure 12). This predicts that at 13, 14, 15, 16 and 17ºC it will take 49, 44, 40, 36 and 33 days, respectively for females to emerge. At 17ºC it took 63 days between the first and second appearance of juveniles and males (one complete life cycle) and at 14ºC therefore it would be predicted that this takes at least another 10 days based on the increased amount of time required for females to emerge at 14ºC.

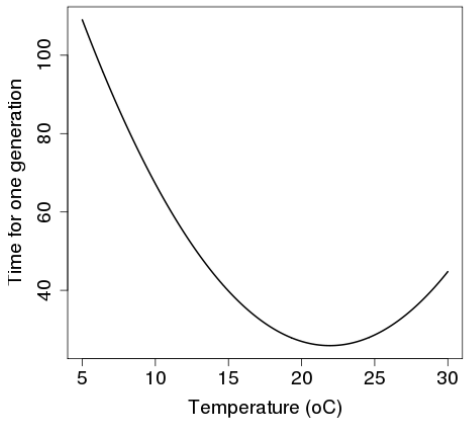


Figure 12 Days required for females of *G. pallida* to first occur on the root surface of Desirée at different temperatures.

iv) *Comparison of* G. pallida *population multiplication at 2 field sites: Harper Adams, England and Luffness, Scotland with and without nematicide treatment*s

Trials were conducted in 2011 and 2012 at 2 field sites; Harper Adams in England and Luffness in Scotland to compare multiplication of PCN during the growing season in 2 geographically separated sites. The sites were infested with *G. pallida* and the population was monitored with monthly soil sampling followed by cyst extraction and qPCR assays to quantify the population level. In 2011, four cultivars were used (Desirée, Maris Piper, Estima and Cara) and in 2012 Estima was replaced with Cara. The plots included 5 replicates with 3 plants for each cultivar in each replicate for each soil collection time point. Results are presented for 2011. The trials in 2012 had low initial Pi at both sites and the trends in multiplication were highly variable.

Figure 13 shows the overall reproductive factor 20 weeks after planting for the 4 cultivars at the 2 sites and with and without nematicide treatment. At Luffness the nematicide treatment (Vydate) resulted in significantly reduced multiplication for all four cultivars whereas at Harper Adams the treatment with Fosthiazate was overall less effective.

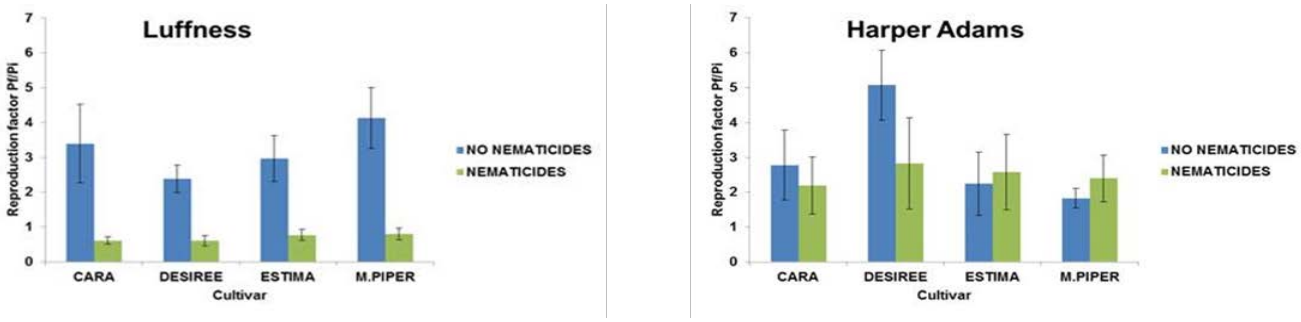


Figure 13 Reproduction factor (Pf/Pi ratio) after the 20 weeks of planting. The bars are standard errors of the means for the each cultivar.

The changes in the populations during the growing season at Harper Adams and Luffness for the 4 cultivars and with and without nematicide treatments are shown are shown in Figures 14-17. Generally a decrease in eggs/gm soil was seen up to 12 weeks after planting with an increase occurring at 16 weeks without nematicide treatment at Luffness whereas at Harper Adams the increase started at week 12. At both sites the increase in the eggs/gm soil occurred later with the nematicide

treatment. The cultivars did not differ at either site in their patterns of population change.

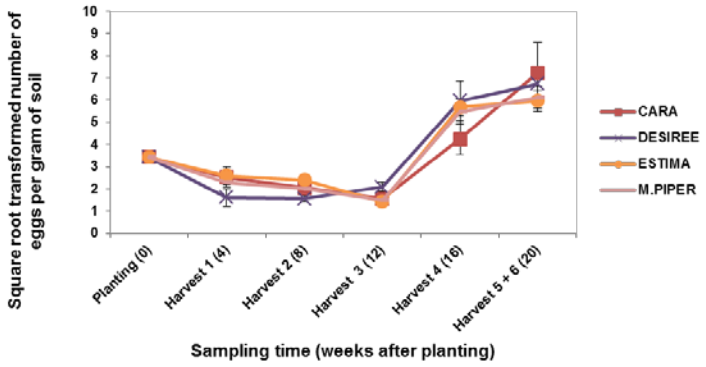


Figure 14 Changes in the *G. pallida* population over the growing season without using nematicides at Luffness. The bars are standard errors of the means for the each cultivar.

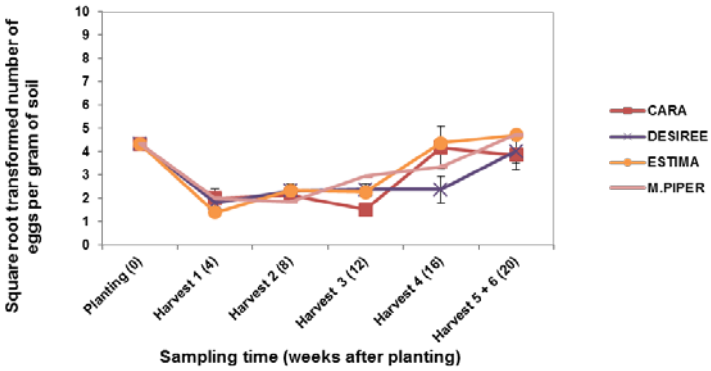


Figure 15 Changes in the *G. pallida* population over the growing season with the nematicides at Luffness. The bars are standard errors of the means for the each cultivar.

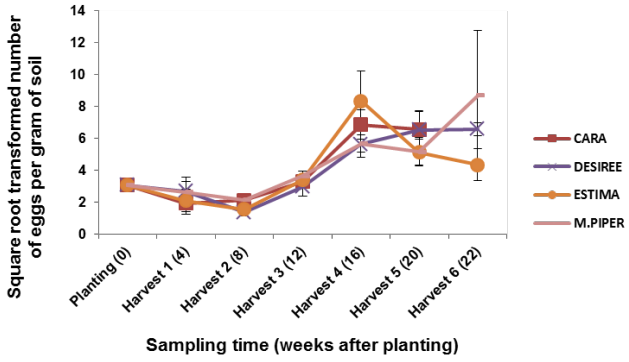


Figure 16 Changes in the *G. pallida* population over the growing season in Harper Adams in square root transformed number of eggs per gram of soil without using nematicides. The bars are standard errors of the means for the each cultivar.

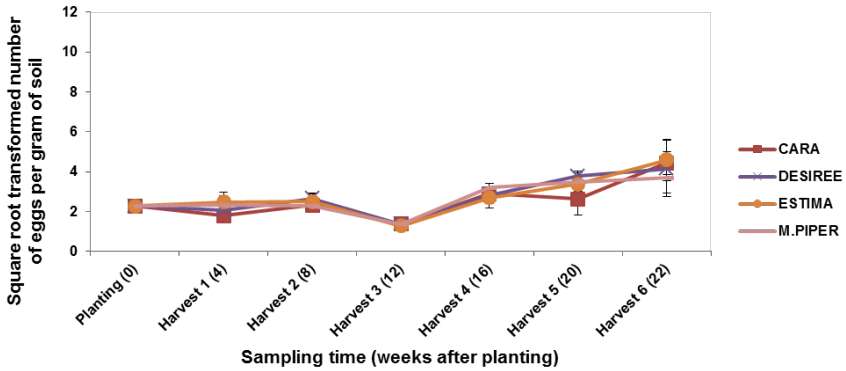


Figure 17 Changes in the *G. pallida* population over the growing season in Harper Adams in square root transformed number of eggs per gram of soil with nematicides. The bars are standard errors of the means for the each cultivar.

The difference between the 2 sites is clear when the data from the cultivars is combined (Figures 18 and 19) with the reduction in eggs/gm soil with the nematicide treatment apparent at both sites but at Harper Adams the increase in the eggs/gm soil occurred earlier than at Luffness.

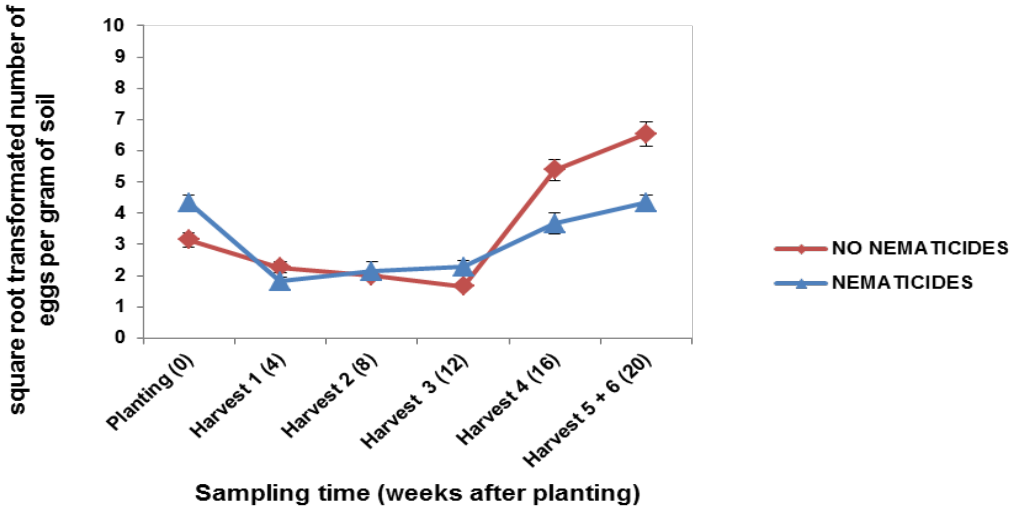


Figure 18 Comparison of the number of eggs/gm soil from untreated and nematicide treated plots over the 20 week growing period at Luffness. The bars are standard errors of the means for the each cultivar.

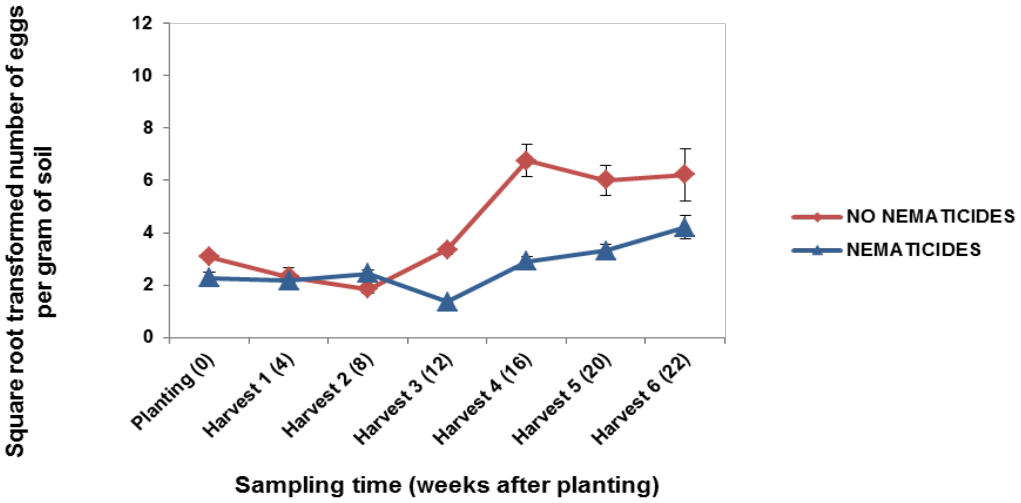
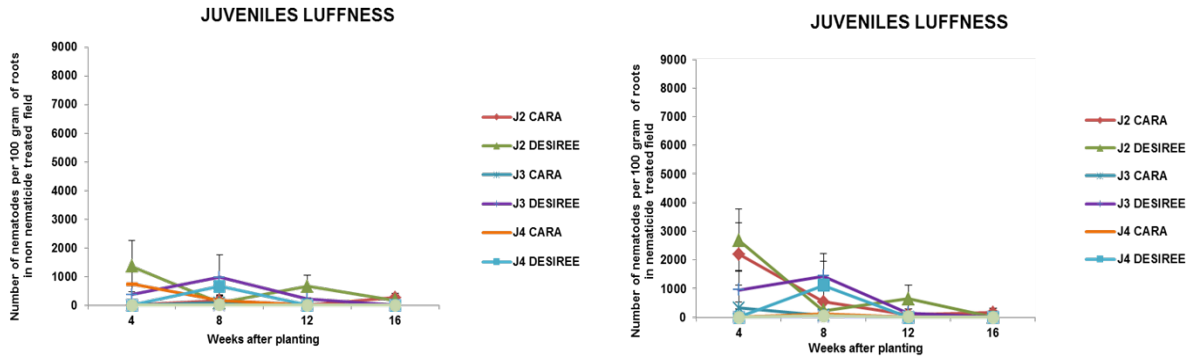


Figure 19 Changes in the population over the growing season for both treated and untreated plots in Harper Adams in square root transformed number of eggs per gram of soil for both treatments. The bars are standard errors of the means for the each cultivar.

Roots of Cara and Desirée were obtained at the time of soil sampling at both Luffness and Harper Adams and these were examined for the presence of developing stages of PCN after staining with acid fuchsin. At Luffness J2, J3, J4 and females were observed in the roots of both cultivars and with and without nematicide treatment. Most females were observed at week 8. Roots were not available after week 16 as they had rotted. At Harper Adams a second incidence of the J2 stage was observed at weeks 20 and 22 in the roots from Cara both with and without nematicide treatment. The maxinum numbers of females were observed at week 8 and a few were observed at week 20 with Cara roots and at week 22 with Desirée both without nematicide treatment. Examples of these visual observations are shown in Figure 21.



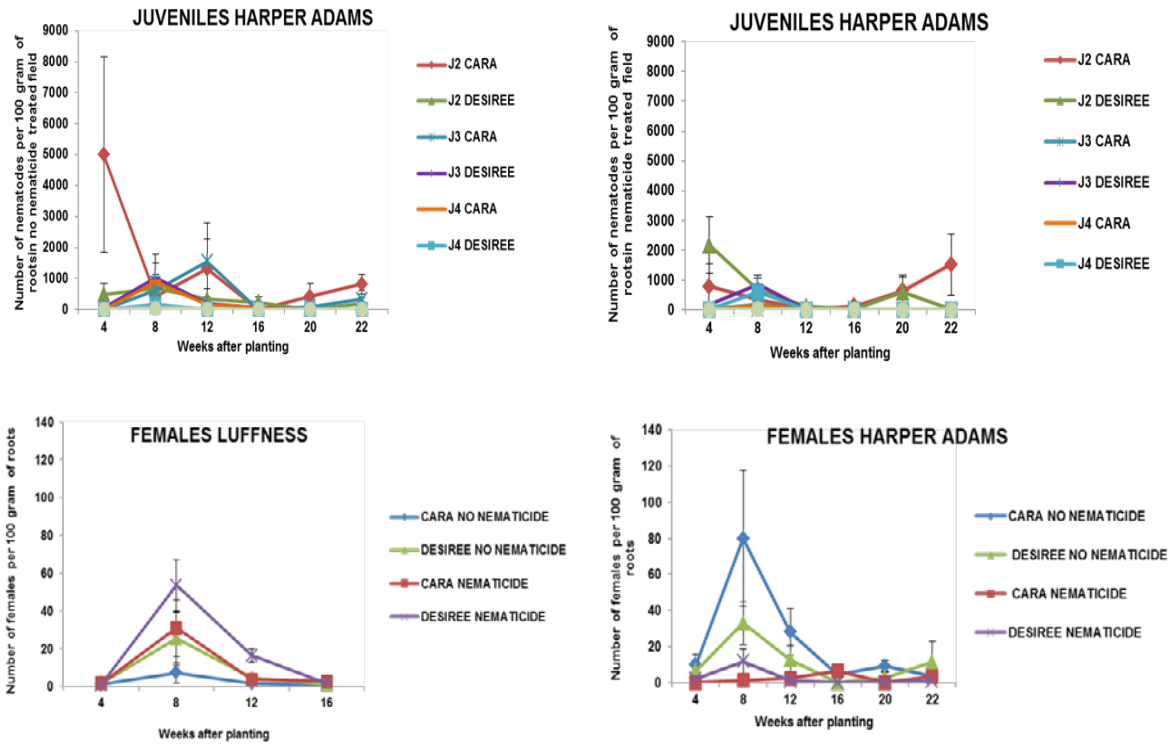


Figure 20 The numbers of J2, J3, J4, and females observed inside acid fuchsin stained roots of cultivars Cara and Desirée from 2011 field trial at Luffness and Harper Adams during the field trial. The bars indicate the standard errors of the mean.

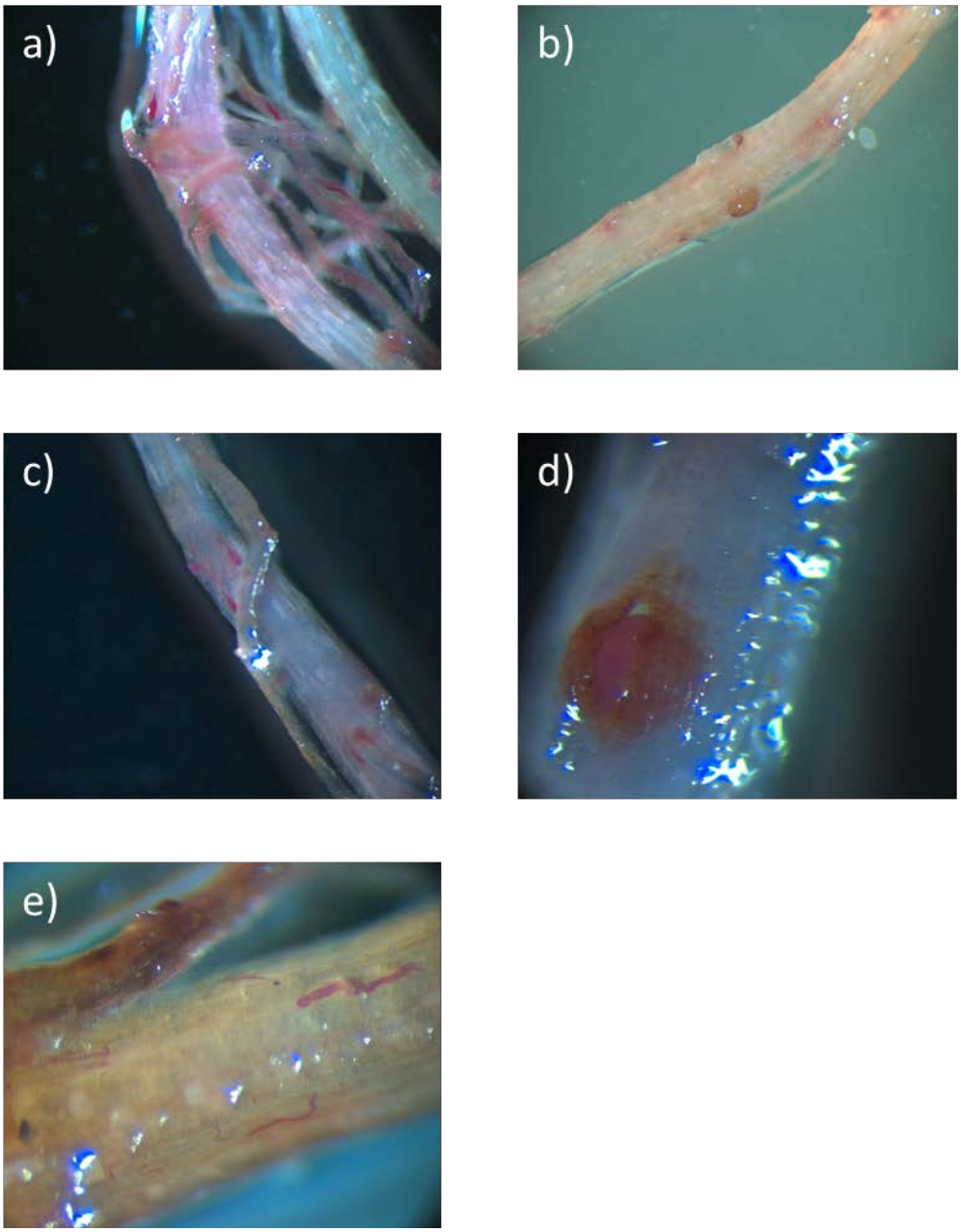


Figure 21 Acid fuchsin stained potato cyst nematodes in potato roots from the field experiments performed in 2011; a) J2 and J4 in roots of cv Desirée 8 weeks after planting in Luffness in nematicide treatment, b) female in roots of cv Desirée 12 weeks after planting in Luffness in nematicide treatment c) in roots of cv Cara 8 weeks after planting in Harper Adams in non nematicide treatment d) female found in roots of cv Cara 8 weeks after planting in Harper Adams in non nematicide treatment, e) J2 and J3 found in roots of cv Cara 22 weeks after planting in Harper Adams in nematicide treatment.

4) Determine if nematodes that complete a second generation on partially resistant potatoes are more virulent

The growth cabinet experiment was conducted to compare development of *G. pallida* on the susceptible Desirée and partially resistant Morag (see above) and to determine if there was a difference in the development of a second generation on the two potato genotypes and in 2 different temperature regimes.

There was a reduction in the total number of cysts produced on cv Morag compared to Desirée over the 16 weeks that the experiment lasted. Also, significantly fewer cysts were produced at the average temperature of 17º compared to 14ºC with Desirée (Figure 22).

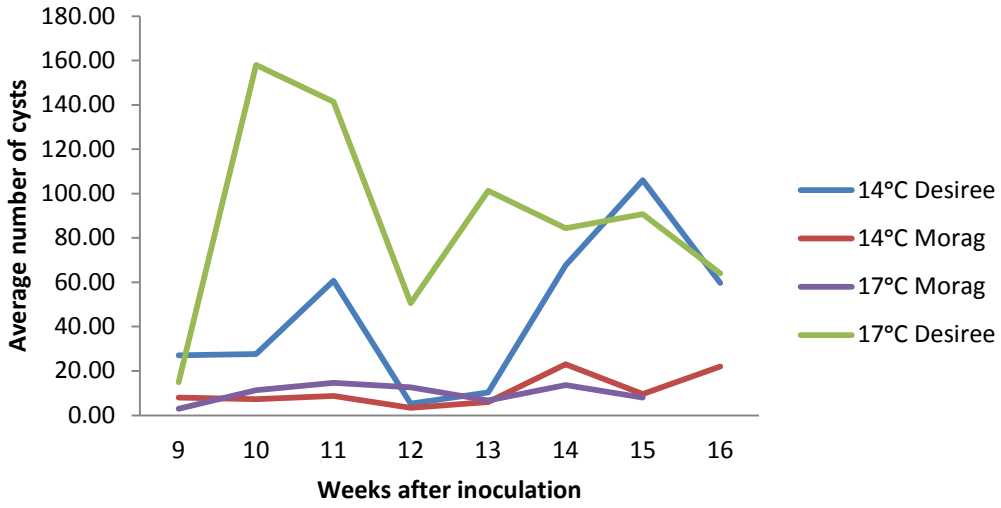


Figure 22 Average number of cysts of *G. pallida* in soil at 9-16 weeks in growth cabinet experiment at average temperatures of 14°C and 17°C with cv Desirée and Morag. No cysts were observed before 9 weeks and the initial inoculum was removed at week 5.

The egg content of cysts collected at week 11 from cv Morag was significantly lower when compared to those from cv Desirée (Figure 23). Differences in the egg content in the two temperature regimes for either cultivar were not significant with a sample of 10 cysts/replicate. The average size and related volume of cysts from cv Morag was also significantly smaller than those from cv Desirée.

The loss of plants during this experiment precluded the collection of cysts to examine the virulence of the second generation from cv Morag. Loss of plants in the second growth cabinet experiment conducted with *G. rostochiensis* also precluded completion of the second generation and the collection of cysts.

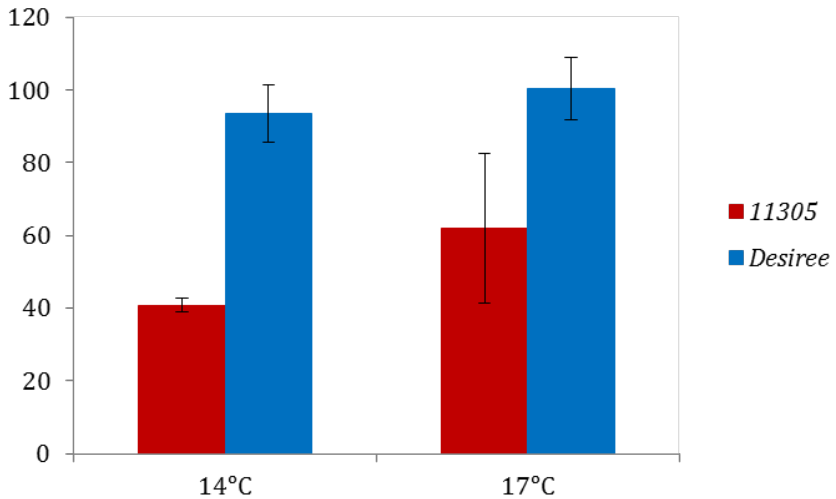


Figure 23 Average number of *G. pallida* eggs per cyst at 14°C and 17°C on cvs Desirée and Morag (c11305) at week 11. The bars indicate the standard error of means.

5) Develop a model to describe the population dynamics of a partial or complete second generation of PCN

A logistic curve Y=A + C/(1 + EXP(-B*(t - M))) was fitted to the hatching data (section 3). The parameters from the logistic curve were plotted as a function of temperature and then curves fitted to these (Figure 24). These curves were used by Helen Kettle of BioSS to produce further equations that can be used to estimate the amount of time required for 50% of hatching to occur with temperatures obtained from the field. For example, soil temperatures recorded in 2011 at 3 hourly intervals from the time of planting at a 20cm depth in potato ridges at Whitewater, Scotland and Harper Adams, England, were used. At Whitewater the temperatures over the growing season varied from 8 to 18.5˚C with a mean of 13.2˚C, whereas at Harper Adams they ranged from 9.5 to 22.5ºC with a mean of 15.4˚C. Using the soil temperature data it was predicted that the sites differed by around 5 days in the time required for half of the hatching to occur. For *G. pallida* it was 16 days at Whitewater but only 11 days at Harper Adams; for *G. rostochiensis* it is 13 days at Whitewater and only 8 days at Harper Adams.

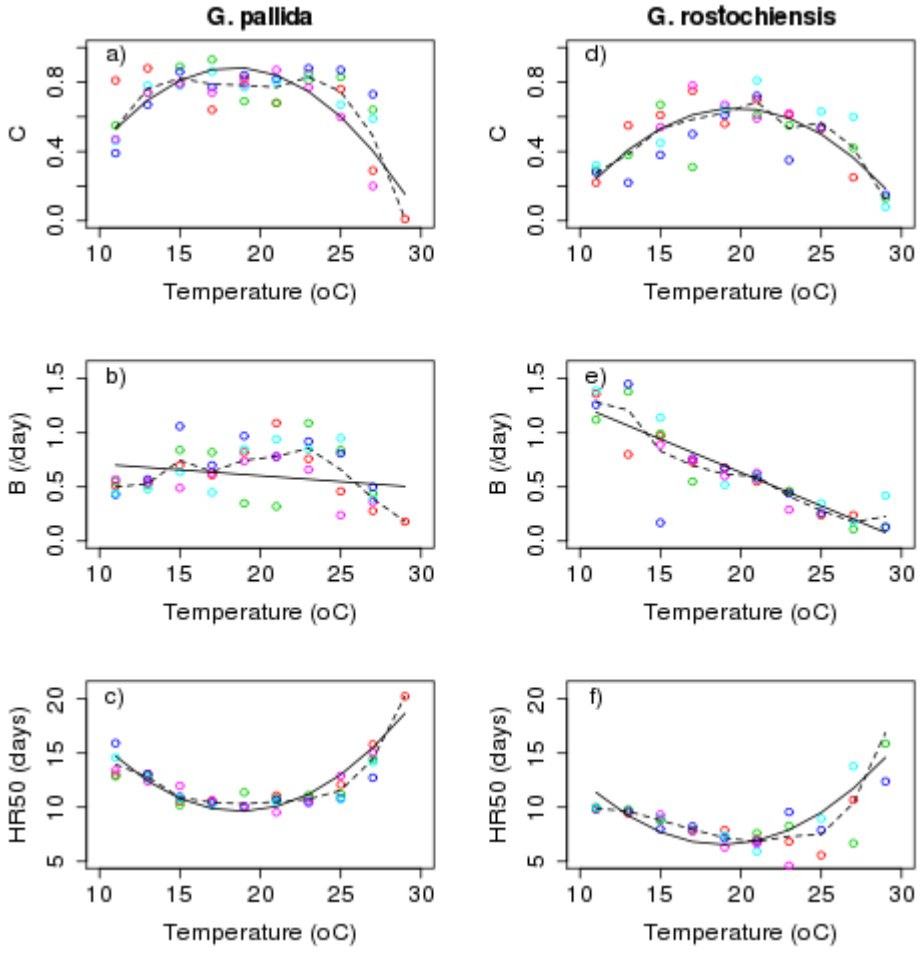


Figure 24 Parameters for the logistic equation (Eq. 1) as a function of temperature. Each replicate is shown by the same coloured dots, the dashed line is the mean of the replicates and the solid line is the predicted curve from Equations 2-4.

The experiments that are described in section 3 have been used by Helen Kettle at BIOSS to develop a dynamic stage-structured simulation model which allows the number of nematodes at each life stage to be evaluated at any moment in time, based on a time-series of soil temperatures. This means that, given the correct parameter values, the number of generations of PCN that can survive and the

number of eggs remaining at the end of the growing season for different soil temperatures can be predicted. An example of the summarised life cycle information that is being used for the model is shown in Figure 25.

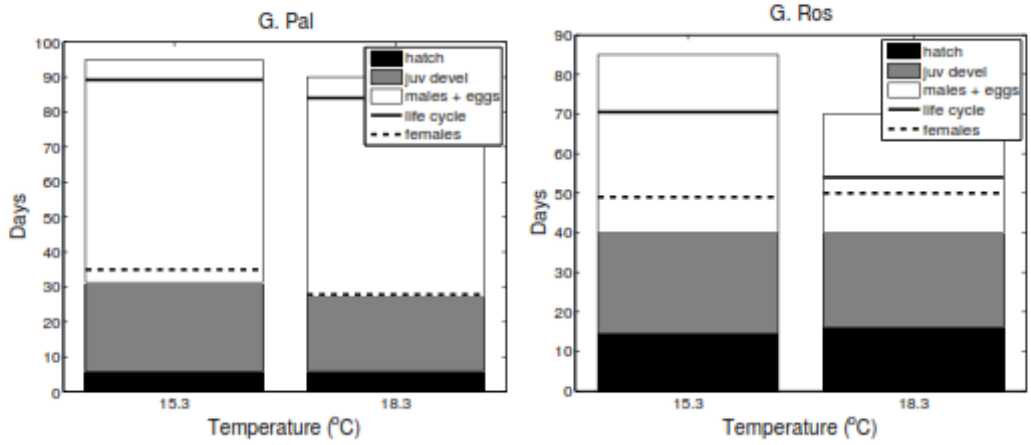


Figure 25 Life stage timings (in days) inferred from growth cabinet data. “Hatch” refers to the time for hatching from the start of the experiment; “juv devel” to the time for juveniles to develop to males (i.e. time between presence of juveniles and males outside of the roots), “males + eggs” is the time from the emergence of free males to the second hatch. The thick horizontal line, “life cycle”, indicates the length of the total life cycle i.e. time between second hatch and first hatch. The dashed line, “females” shows when females first emerge.

6) Soil temperatures in potato drills at different sites in the UK

Soil temperatures were obtained at 20cm in potato drills from the start until the finish of the growing season at different sites around the UK and an example of this data is shown in Table 1. This data is needed to understand the variation in soil temperatures around the UK and between seasons and so it can be related to the experimental data that has been obtained concerning the life cycle of PCN in relation to temperature and for use in the model relating temperature and the PCN life cycle. Significant differences in soil temperatures are likely to be an important factor in the amount of PCN multiplication within a growing season.

For example, the implication for hatching at 2 sites that differed in their temperature profiles in 2011, Harper Adams, England and Whitewater, Scotland was assessed based on the hatching profiles that were produced over the temperature gradient (section 3). At Harper Adams hatching was predicted to be faster for both species than at Whitewater in Scotland with *G. rostochiensis* hatching more quickly than *G. pallida,* however, the amount of hatching was predicted to be greater for *G. pallida* at both sites.

Figure 26 shows plots of the soil temperatures in the 2 trial sites at Harper Adams and Luffness in 2011 and the prolonged period of warmer temperatures at Harper Adams towards the end of the growing season. The mean soil temperature over 150 days of the growing season at Luffness was 14.15°C and at Harper Adams 14.85°C

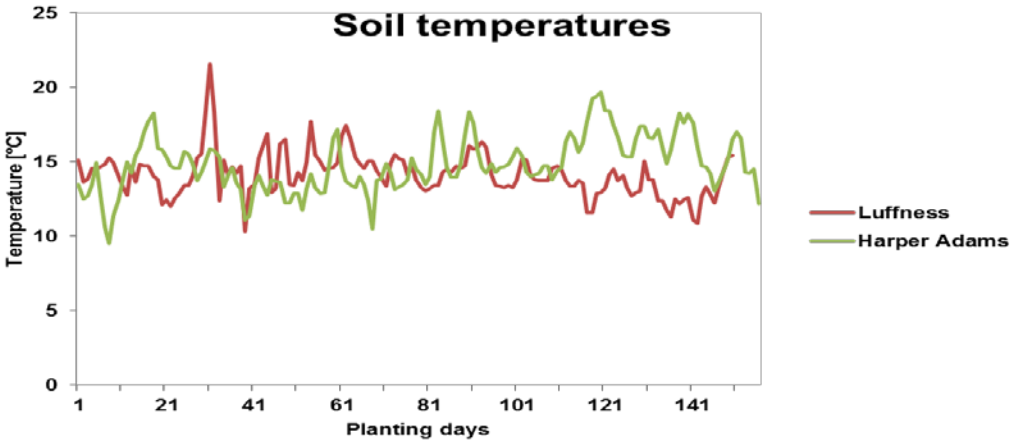


Figure 26 Soil temperatures in the potato drills of the field trials in Harper Adams and Luffness in 2011 at 20cm over growing season.

The highest average soil temperature was obtained at Luffness in 2010 (17ºC) and the lowest was obtained at Harper Adams in 2012 (13.68ºC) excepting the temperatures in Jersey. A trend with increasing temperature and decreasing latitude can occur in some years (2011) and some years the temperatures are significantly lower (2012) than others (2010). The temperatures in 2011 were generally low but many sites had average temperatures in 2010 and 2012 >15ºC.

Warmer soil temperatures not only increase the rate of hatching for both species but also increase the total amount of hatching leading to increased population levels on susceptible hosts and damage to the crop. Therefore, regions of the UK with relatively higher soil temperatures, or years in which crop planting coincides with warmer soil temperatures are thus more likely to have higher levels of hatching of PCN and thus greater multiplication and have greater challenges in controlling population levels. Low soil temperatures are likely to favour *G. pallida* whereas warmer temperatures are likely to favour *G. rostochiensis,* which hatches more quickly. This has implications for interspecific competition between the 2 species at different temperatures when they occur as mixtures in the field, the host response to mixed infections and the composition of the final PCN populations.

	Country	Planting and harvesting dates	Max	Min	Average
Year 2010					
Luffness, Millfield*	Scotland	27/5/10 1/10/10	22.5 22/06/10	7.5	17.00
Luffness, Forefield 2*	Scotland	27/5/10 1/10/10	23.5 5/06/10	7.0	15.61
Luffness, Forefield 1*	Scotland	27/5/10 1/10/10	21.5 5/06/10	7.0	15.63
Year 2011					
Whitewater 1	Scotland	19/5/11 14/9/11	27.5 30/09/11	8	14.64
Whitewater 2	Scotland	19/5/11 14/9/11	27 30/09/11	8	14.51
Balruddery1	Scotland	19/5/11 27/9/11	26.5 30/09/11	6	14.53
Balruddery2	Scotland	19/5/11 27/9/11	27 30/09/11	6	14.60
Luffness 1	Scotland	4/5/11 28/9/11	27.5 3/06/11	8.5	14.14
Luffness 2	Scotland	4/5/11 28/9/11	28.5 3/06/11	9	14.14
Harper Adams JC 1	England	21/4/11 21/9/11	22.5	9	15.38
Harper Adams JC 2	England	21/4/11 19/9/11	22.5	9.5	15.10
Harper Adams 1	England	4/4/11 8/9/11	23.5 23/08/11	8	15.39
Harper Adams 2	England	4/4/11 8/9/11	24 3/08/11	7	14.83
Tetbury 2, Gloucestershire	England	14/4/11 2/8/11	24.5 28/7/11	10	15.05
Kings Caple 1, Herefordshire	England	14/4/11 23/8/11	22.5 3/6/11	10.5	15.99
Kings Caple 2, Herefordshire	England	14/4/11 23/8/11	27.5 3/6/11	9	16.07
Bold Farm 1, Lancashire	England	31/5/11 26/9/11	26 3/6/11	13	15.91
Bold Farm 2, Lancashire	England	31/5/11 26/9/11	24 3/6/11, 4/7/11	13	16.03
Year 2012					
Jersey St Peter's	Jersey	18/1/12 (14/3/12-15/5/12)	30.9 (13/5/12)		11.73
Jersey Trinity	Jersey	27/2/12 (14/3/12-9/7/12)	22 (23/6/12)		11.73
Elgin 1	Scotland	10/5/12 20/9/12	24.5 27/5/12	7	14.48
Elgin 2	Scotland	10/5/12 20/9/12	23 27/5/12	6	13.70
Harper Adams	England	2/4/12 4/10/12	36.5 (23/5/12)	4.5	13.68
AB Roadside 1	England	3/5/12 13/8/12	23.6 (24/7/12)	7	15.52
AB roadside 2	England	23/5/12 3/10/12	23.6 (24, 25/7/12)	?	16.23

Table 1 Soil temperatures were obtained at 20cm in potato drills using a DS1920-F5 Temperature ibutton (HomeChip, Milton Keynes, UK). The date of planting and harvesting, the maximum and minimum and average temperatures in 2010, 2011 and 2012 are shown for different sites in the UK.

g) Composition of field populations

Potato cyst nematodes were introduced into Europe from S. America and three possible sites of origin for the *G. pallida* introductions have been identified near Lake Titicaca in Peru. These three introductions can be distinguished based on the mitochondrial cytochrome B (cytB) sequence. In the UK, three introductions have been identified of *G. pallida* though Pa1 has only been reported from Duddingston in Scotland. Single cysts from the field trial sites in Harper Adams and Luffness as well as several other sites in East Lothian were used in a phylogenetic analysis based on cytB. DNA was extracted from cysts, a PCR performed using cytB primers and the product sequenced. A phylogenetic tree is shown in Figure 27 which includes 3 sequences from *G. mexicana* and sequences of cytB from *G. pallida* from Peru. The cysts from Harper Adams and Luffness fall into 2 groups which include European and Peruvian populations. This demonstrates that fields in England and Scotland contain 2 introductions of *G. pallida*. The third group contains the Pa1 population and 1 cyst from East Lothian was in this group. This indicates that the Pa1 pathotype is unlikely to be localised just to Duddingston.

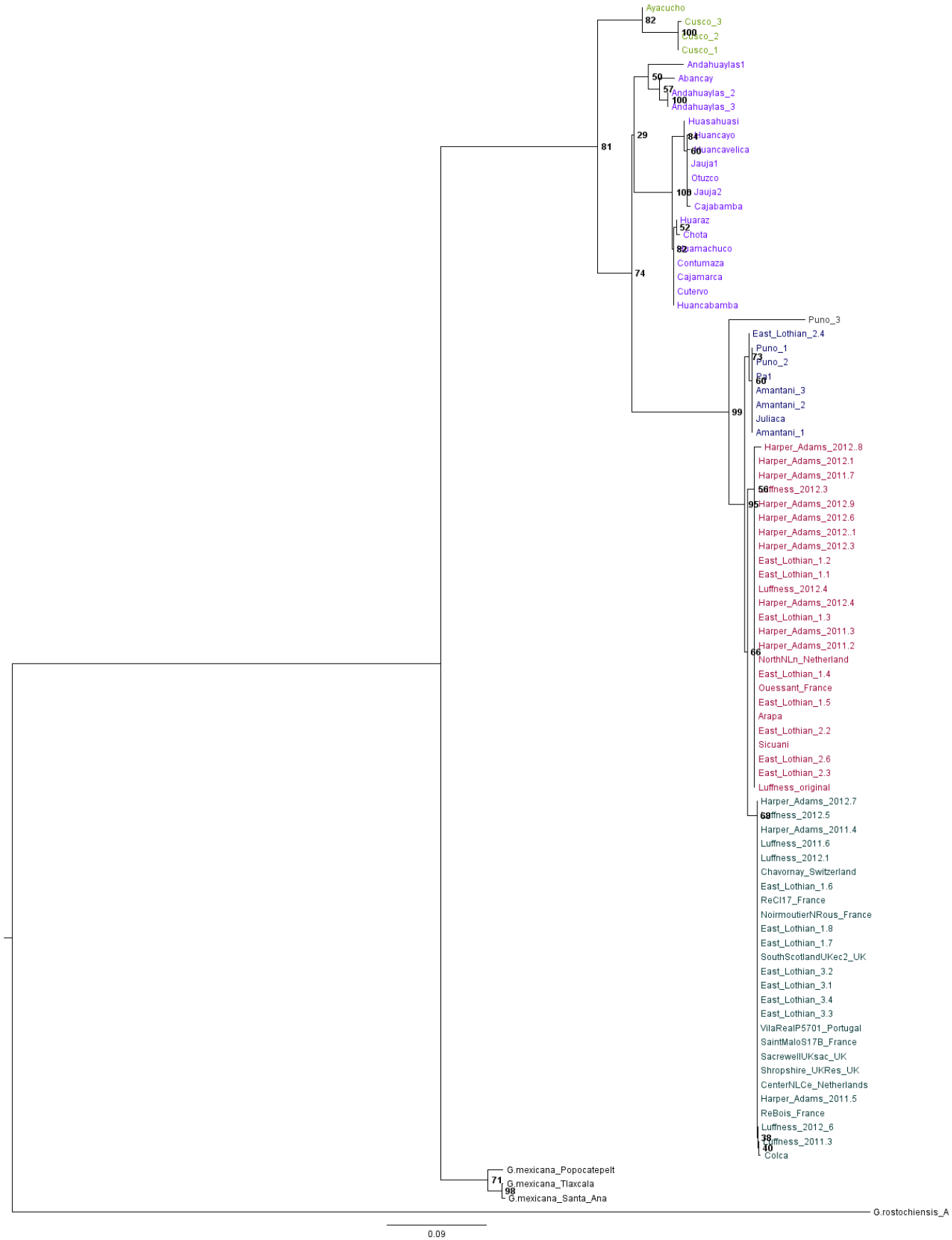


Figure 27 Phylogenetic tree showing the relationship between cytochrome B sequences obtained from individual field cysts from Harper Adams, England and Luffness, Scotland with other sequences obtained from sites in Europe and Peru. A cyst from East Lothian is shown in the group with Pa1. Three *G. mexicana* sequences were used as outgroup and the boot strap values are shown.