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Population dynamics of potato cyst nematodes in relation to temperature

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Pracę dedykuję mojej mamie oraz Małgosi z Irkiem i Dorocie, bez wsparcia których nigdy by ona nie powstała.

DECLARATION

This thesis is my own composition. The results presented here are of investigations conducted by myself. Work other than my own is clearly indicated with references to relevant researchers and/or their publications. This work has not, in whole or in part, been previously presented for a higher degree.

The research was carried out at The James Hutton Institute, Invergowrie, Scotland, under the supervision of Dr Vivian C. Blok, Dr M. Finlay B. Dale and Prof. Paul R. J. C. Birch.

Agata Monika Kaczmarek

STATEMENT

I certify that Agata Monika Kaczmarek, a candidate for the degree of Doctor of Philosophy in the University of Dundee, has fulfilled the relevant Ordinance and Regulations of the University Court, and is qualified to submit this thesis.

Dr Vivian C. Blok

The James Hutton Institute

Prof. Paul R. J. C. Birch

University of Dundee

PUBLICATIONS ARISING FROM THIS WORK

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Kettle H., Kaczmarek A., and Blok V. C., 2014. Modelling the Population Dynamics of Potato Cyst Nematodes. (in preparation)

ABBREVIATIONS

ANOVA	Analysis of variance
BioSS	Bioinformatics and Statistics Scotland
CV	Cultivar
CytB	Cytochrome B
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic acid
EPPO	The European and Mediterranean Plant Protection Organization
EU	European Union
FAA	Formalin-Acetic Alcohol
Ha	Hectare
HPLC	High-Performance Liquid Chromatography
IAA	Indole-3-acetic acid
ITS	Internal transcribed spacer
J2	Second stage juvenile
J3	Third stage juvenile
J4	Fourth stage juvenile
JHI	The James Hutton Institute
Kg	Kilogram
Luff	Luffness
Min	Minutes

mt DNA	Mitochondrial DNA
PCL	Potato Council
PCN	Potato cyst nematode
RFLP	Restriction fragment length polymorphism
Pf	Final population
Pi	Initial population
PRD	Potato root diffusate
PVPP	Poly(vinylpolypyrrolidone)
qPCR	Quantitative polymerase chain reaction
qty	qPCR quantity value
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
RT-PCR	Reverse-transcription polymerase chain reaction
SASA	Science and Advice for Scottish Agriculture
SCRI	Scottish Crop Research Institute
SDW	Sterile distilled water
SNP	Single nucleotide polymorphism
TBE	Tris Borate EDTA
UV	Ultraviolet

ABSTRACT

Population dynamics of potato cyst nematodes *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida (*Stone) and their interactions with potato plants are not sufficiently described to explain changes in population dynamics and yield reduction among seasons and locations. This thesis describes experiments to clarify the mechanisms of PCN population increase and associated damage to potato primarily in relation to temperature. The research was undertaken in controlled environments and in the field to examine the impact of temperature and mixed populations on the life cycle and population dynamics of potato cyst nematodes. The data will be used to develop a model that relates the PCN life cycle with temperature. Soil temperature data were also obtained from different UK locations within potato drills during the growing season to provide a context for the model. Results from this thesis indicate that both species of PCN, *Globodera rostochiensis* and *G. pallida*, are likely to increase more rapidly and in greater numbers with susceptible cultivars if soil temperatures increase in the future and regions of the UK with relatively higher soil temperatures are more likely to have higher and faster population multiplication of PCN.

1. GENERAL INTRODUCTION

The potato cyst nematodes *Globodera rostochiensis* (Wollenweber) and *G. pallida* (Stone) Skarbilovich are major parasites of potatoes and other members of the *Solanaceae* family and are the largest constraint by a pest on potato production in the UK. The survey done by Minnis *et al.* in 2002 revealed that both species were present in UK potato land and 64% of 484 potato fields in England and Wales sampled in 1997 and 1998 were infested with PCN. Of these, 67% were infested with *G. pallida* only, 8% with *G. rostochiensis* only, and 25% with mixed populations of the two species. The dominance of *G. pallida* infestations in England and Wales is expected to increase due to the continued cultivation of *G. rostochiensis* resistant potato cultivars, such as Maris Piper, which favors the selection of *G. pallida* over *G. rostochiensis* in the field (Trudgill *et al*., 2003).

1.1. Introduction and distribution of PCN in Europe

The potato cyst nematodes originate from South America and were introduced into Europe in the middle of nineteenth century (Evans *et al.,* 1975). The Andes are considered to be the original home for PCN (Evans *et al.,* 1975; Mai, 1977). The first report of cyst nematodes was published by Kühn (1881), who observed nematodes attacking potatoes and described it as a sub-race of the beet-cyst nematode *Heterodera schachtii.* In 1923, Wollenweber proposed a new species and named it *Heterodera rostochiensis.* This new species was accepted in 1940 when Franklin distinguished it by describing morphological differences between potato cyst nematodes and other cyst nematodes. *Heterodera pallida,* the

second species of potato cyst nematodes, was described by Stone (1972). Skarbilovich (1959) established the subgenus *Globodera* which was later raised to generic status by Behrens (1975) and Mulvey and Stone (1976).

The routes in which PCN was introduced into Europe are still under consideration. It is believed that the cysts were probably transported into Europe together with the breeding material brought in order to find a new source of resistance to *Phytophtora infestans* the cause of potato late blight (Mai, 1977). According to Turner *et al.* (1998) and Hockland *et. al*. (2012), one of the routes by which PCN has been spread is with seed potatoes and subsequently have been exported to different continents from Europe, which has become a secondary distribution source. Most pathways of spread are caused by passive transport and leaks in hygiene e.g. contamination of breeding material and farm saved seed, transportation of cysts on boots, tractor tires or introduction of soil from an infected field (Turner and Evans, 1998).

PCN have been recorded in almost all potato producing countries (EPPO, 2014) (Figure 1.1). According to EPPO, PCN have been detected in 71 (*G. rostochiensis*) and 44 (*G. pallida*) countries (EPPO, 2014). Over time, however, the proportions of the two species have changed due to the use of *G. rostochiensis* resistant cultivars. For example in the United Kingdom the dominant species previously was *G. rostochiensis* but *G. pallida* has become more prevalent (Turner and Evans, 1998). Based on the survey done by Minnis *et al.* (2002)showed an increase in the incidence of PCN since a previous survey (Hancock, 1996) and confirmed the perceived shift towards *G. pallida* as the dominant species.

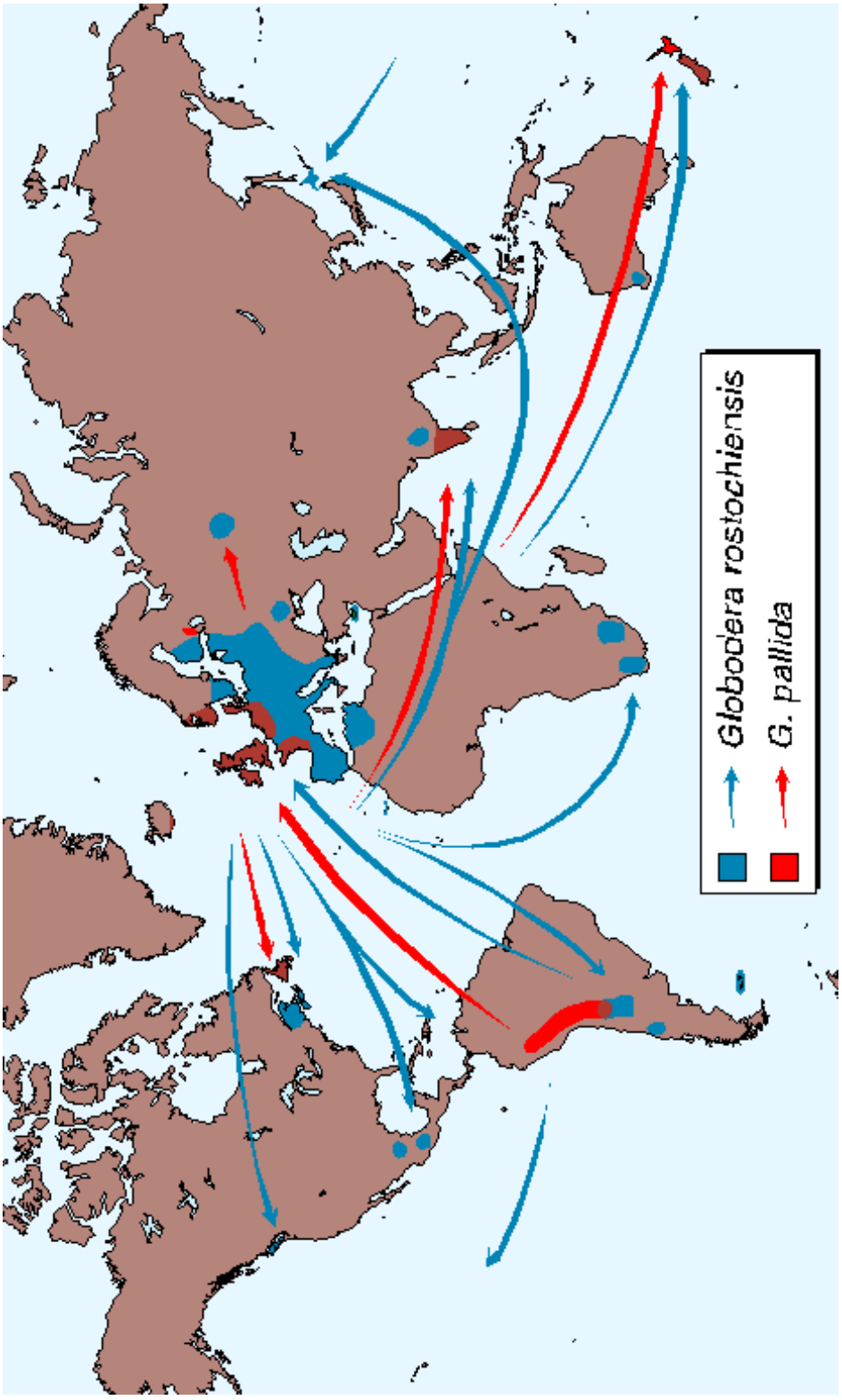


Figure 1.1 World spread of *Globodera rostochienisis* (blue) and G*. pallida* (red) based on Turner and Evans (1998).

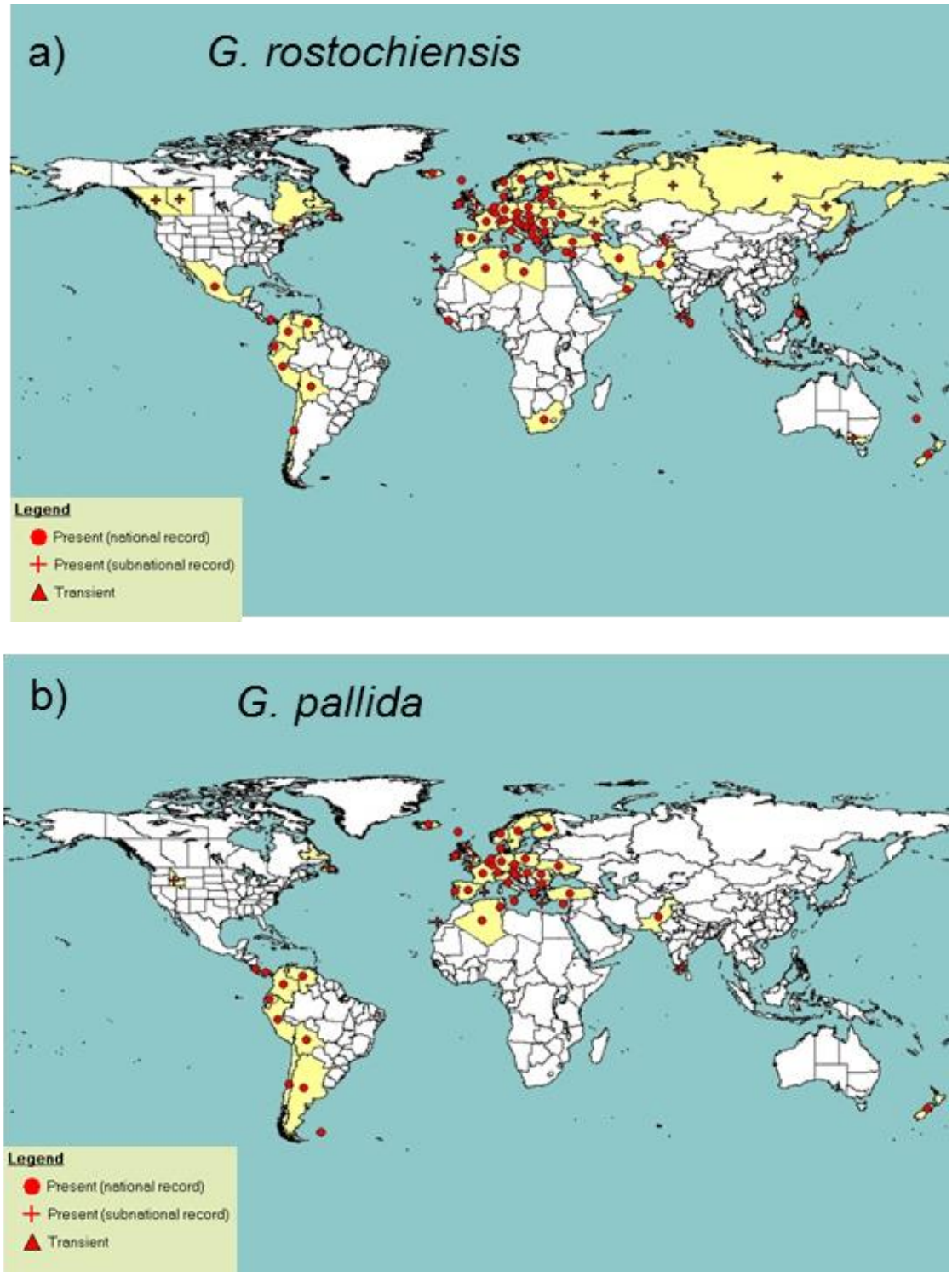


Figure 1.2 World distribution of *Globodera rostochienisis* (a) and G*. pallida* (b) based on EPPO (2014). Countries with PCN present are marked in yellow.

1.2. Life cycle of the potato cyst nematodes

The life cycle of potato cyst nematodes (PCN) is initiated by exudates produced from a host's roots. Following hatching and invasion of the root, PCN creates an intimate relationship with its host via the induction of a complex structure, a feeding site otherwise known as the syncytium, in the vascular cylinder of the potato roots (Rawsthorne and Brodie, 1986).

The host roots secrete factors which stimulate dormant eggs within the cyst to hatch. The juveniles emerge and move through the soil, attracted to the host by the root exudates. The second stage juveniles (J2) puncture and then penetrate the roots behind the growing tips, and following a short migration period within the root, each J2 selects a cortex cell for inducing a syncytium (Turner and Evans, 1998). This is the pathogenic stage during which the initial feeding cell soon expands to form an elaborate syncytium which transfers nutrients from the vascular tissue to the feeding nematode (De Boer *et al*., 1996). Syncytium induction involves proliferation of the endoplasmic reticulum, ribosomes, mitochondria and plastids in the cell, and enlargement of the nucleus without mitosis, a phenomenon known as endoreduplication (Gheysen and Fenoll, 2002). The reduction in transpiration rates and other physiological functions of the host resulting from damage to the root system as a result of the nematode infection can substantially decrease yields (Schans and Arntzen, 1991).

Males do not feed once they have emerged from the host. The sex of PCN is environmentally determined by the value of available nutrition and temperature (Franco 1979; Kakaire *et al*., 2012; Schmidt *et al.,* 1993; Trudgill *et*

al., 2005; Van der Waals *et al*., 2013). When food supply is limited, males predominate as they are able to develop under conditions of nutrient stress and require much less food than females. Males leave the roots 4 to 5 weeks after the initial invasion whereas the sedentary females continue to feed for 2 to 3 months. The female body swells, ruptures the plant's root surface but it continues to feed from the syncytium via the head and neck which remain attached, buried inside the root (Franco, 1986). Before leaving the root, males regain their original eelworm-like shape. When they emerge from the root, they locate and mate with females, which then fill with fertilized eggs and subsequently die. The dead female becomes a brown, hard cyst, which may contain from a few to up to 200-500 eggs (Turner and Evans, 1998) (Figure 1.2 and Figure 1.3).

Once the J2 have developed inside the cyst, they generally enter an extreme form of dormancy, known as diapause Nematodes in diapause do not hatch in response to host cure. Diapause is broken by exposure to a period of cold plus in therefore an overwintering strategy. (Turner and Evans, 1998).

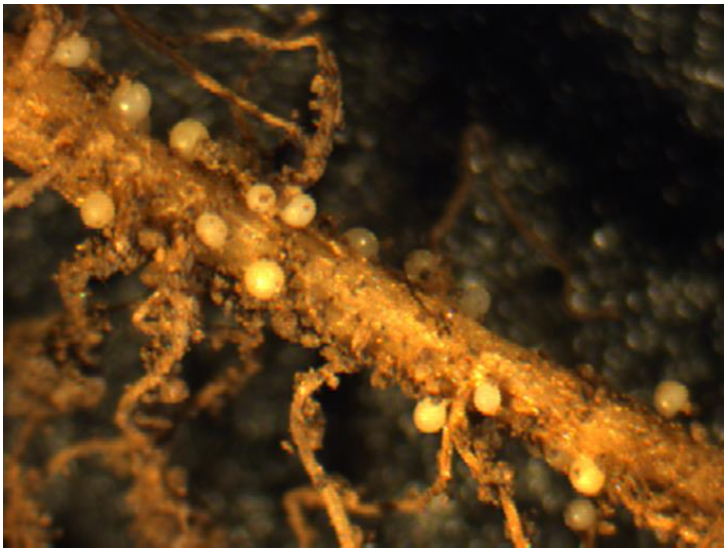


Figure 1.3 Females of potato cyst nematodes *Globodera pallida* found on roots of cv Desirée during the field experiments at Harper Adams in 2011.

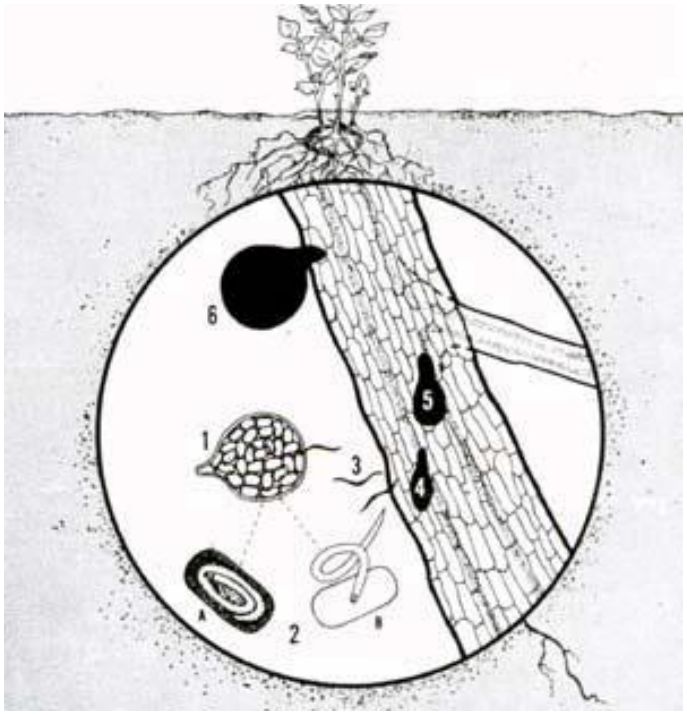


Figure 1.4 Life cycle of *Globodera* spp. 1. Juvenile (J2) in egg (2A), J2 hatching from egg (2B), juveniles entering the potato root (3), J3 and J4 stages inside root (4 and 5), adult female (6) (Pickup, 2002).

1.3. Diagnostics and pathotyping of PCN

Current diagnostics for the potato cyst nematodes combine morphological with molecular analyses. There are several methods available for extracting potato cyst nematodes from soil, including juvenile stages as well as cysts (EPPO 2013). Traditional diagnostic methods are based on morphometric measurements of the differences in the cyst's perineal area, i.e. Granek's ratio (the distance from the anus to the nearest edge of the vulval basin, divided by vulval basin diameter) and the number of cuticular ridges between vulva and the anus. The main distinguishing characters of the second-stage juveniles are stylet length and stylet knob shape, but these are not completely unique giving rise to some ambiguity with the use of these characters for identification. Also, morphological identification requires skilled taxonomists and nowadays individuals with appropriate training are becoming rare, morphological identification is low throughput and it is relatively expensive and time consuming (Fleming and Powers, 1998). Molecular techniques are therefore becoming more frequently used. Several DNA-based molecular diagnostics have been developed for routine application in phytosanitary laboratories (see reviews: Powers, 2004; Blok, 2005; Reid *et al.,* 2010) which are reliable and robust. For phytosanitary laboratories, the European and Mediterranean Plant Protection Organization (EPPO, 2014) publishes recommendations to its member countries. Current recommendations for *Globodera rostochiensis* and *G. pallida* are included in Diagnostic Protocol PM 7/40(3) (EPPO, 2014).

In 1977 Kort *et al*., proposed a scheme to classify pathotypes of PCN in terms of their multiplication on cultivars with different resistance genes. A pathotype

was characterized by the reproduction rate (Pf/Pi ratio). To establish the Pf/Pi with pot experiments (Kort *et al*., 1977) or in Petri dishes (Mugniery *et al*., 1989; Janssen, 1990), plants are inoculated with known numbers of cysts (Pi) and the number of newly developed cysts (Pf) is determined. Potato genotypes with the *H1* resistance gene differentiate the Ro1/4 pathotypes of *G. rostochiensis* from Ro2 and Ro3, which H2 distinguishes the Pa1 pathotype of *G. pallida* from Pa2/3. The pathotypes Ro2, Ro3 and Ro5, and Pa2 and Pa3 are differentiated by quantitative resistance. However, multiplication rates may not always be an appropriate measure for pathotyping (Janssen, 1990) due to density dependence (Forrest and Phillips, 1984; Rawsthorne and Brodie, 1986, Trudgill, *et al.,* 2014). Also, variation in multiplication is affected by environmental factors (Franco, 1979; Schmidt *et al.,* 1993; Trudgill *et al.,* 2005; Kakaire *et al*., 2012; Van der Waals *et al*., 2013). Nijboer and Parlevliet (1990) suggested some changes in the classification of pathotypes within *G. rostochiensis* and renamed the pathotypes into 3 groups: Ro1 (Ro1 and Ro4), Ro3 (Ro2 and Ro3) and Ro5 (Ro5). Within *G. pallida* the distinction of Pa2 and Pa3 remains questionable. Phillips and Trudgill (1998) discussed that a more realistic method should be used to assess the relative virulence of different PCN populations which would display a broad variation in virulence within the pathotype.

Picard *et al.* (2007) and Plantard *et al.* (2008) suggested that *G. pallida* could be subdivided into 5 clades based on molecular markers. These clades mapped to five geographic locations in Peru which are the natural habitat for populations of PCN. Populations found in Europe were clustered together in the same clade with south Peruvian populations from the area near Titicaca Lake. Partial

sequence of cytochrome B and seven nuclear microsatellite loci were used as the genetic markers in these studies. These phylogenetic studies provide an insight into the genetic diversity in *G. pallida* and origins of European populations. However, despite all the difficulties with the scheme established by Kort *et al*. (1977) is still in use in many laboratories (Hockland *et al.,* 2012) because pathotype-specific molecular markers have not yet been developed.

1.4. Economic importance of PCN

Potato cyst nematodes are major pests of the potato crop causing significant economic losses. Infected plants usually have a smaller root system, which explores a smaller volume of soil (Trudgill, 1986). Roots damaged by PCN have a reduced capacity to take up nutrients from soil, they are adversely affected by water stress and have disturbances of nutrient metabolism. In the UK, the direct and indirect crop losses caused by PCN have been valued at 9% of yield annually (Evans, 1993). The economic cost of PCN to the UK potato growing industry was estimated at more than £43M in 1998 based on lost yield alone (Kerry *et al.,* 2002). This value accounts only for direct costs; indirect costs include expenses for fertilizers and irrigation which are used to compensate for poor crop performance due to PCN infestation (Kerry *et al*., 2002). The direct costs of managing a field infected by PCN also includes expenses for pre-plant sampling and nematicides. These costs have increased due to the new PCN Directive (2007/33/EU), which requires increased pre-plant soil testing for all seed crops. In fields where PCN are detected, the directive prohibits growing of seed potatoes and ware may only be grown under an officially approved control

programme which includes using resistant varieties, nematicides or other control measures and rotation (Hockland *et al*., 2000).

1.5. Control options for potato cyst nematodes

Once established, potato cyst nematodes are difficult if not impossible to eradicate due to the persistence of viable eggs in the cysts for many years (Franco, 1986). Despite, the fact that both species of PCN are designated as quarantine pests and thus regulated by legislative control in most potato growing countries, they continue to be spread of, into non-infested land. Most control methods aim to limit the spread and minimize damage from, PCN. When various control methods including prevention (hygiene), crop rotation, chemical and biological control and the use of resistance and trap crops are combined, they can be effective in integrated pest management programmes and can be further combined with components of crop production (e.g. irrigation, fertilization). The most important goals are to prevent the spread of PCN into new areas and to keep population densities within acceptable levels within the context of a profitable potato production system.

1.5.1. Resistant and tolerant cultivars

The use of resistant cultivars to reduce potato cyst nematode populations can be an effective strategy, as shown by successful control of *G. rostochiensis* with the widespread use resistant varieties. The use of these cultivars, such as Maris Piper and Cara, has greatly decreased the abundance of *G. rostochiensis*. These and other *G. rostochiensis* varieties contain the *H1* gene from *S.*

tuberosum ssp. andigena or *Gro1* gene from *S. spegazzinii*. Numerous sources of resistance to the *G. pallida* have been studied (*S. vernei* (Grp1_QTL), *S. multidissectum* (H2), *S. tuberosum ssp andigena* (H3)) (Dalamu *et al.,* 2012). However, no single major resistance gene to *G. pallida* has been identified for use in breeding, and producing cultivars with high levels of resistance has been much harder to achieve. Some varieties are available with partial resistance to *G. pallida (*for example Morag with resistance from *S. vernei* CPC 2488 and CPC 2487 or Vales Everest with resistance from *S. tuberosum spp. andigena* CPC 2802*).* Partially resistant cultivars are not generally in wide demand from the market and therefore are only grown on about 8% of the potato land in the UK.

Tolerance is an ability of plants to yield despite pest attack (Evans and Haydock, 1990). In practice it means that some potato varieties can produce more tubers than others in infested soil. Dale *et al*. (1988) presented results showing significant differences between genotypes of potato in their tolerance to the white potato cyst nematode, although they did not observe a clear association between tolerance and yield potential in the absence of potato cyst nematodes or in the level of resistance to them (Dale *et al*., 1998). Tolerance is a complex character and many factors may contribute including root size and growth rate.

Before choosing a potato cultivar it is crucial that growers have their fields tested to determine PCN population density and identify the species of PCN that are present. Moreover it is important that the same potato cultivar with partial resistance is not planted in consecutive years due to the danger of

adaptation and selection of more virulent populations or different pathotypes or even genetically distinct strains. Ideally, varieties with different resistance genes should be used in rotations (Haydock *et al*., 2010).

1.5.2. Crop rotation

Crop rotation is the most widely practiced control method (Franco, 1986). The are two main principles for crop rotation: first is the reduction of PCN populations levels to allow the subsequent crop to grow and yield; and the second is the protection of competitive, antagonistic and/or predacious nematodes and other organisms at population densities that permit controling of the potato cyst nematodes (Nusbaum and Ferris, 1973). The effectiveness of a crop rotation is dependent on different factors such as the species of PCN present and their initial population level, soil type and cultivars in previous rotations and the decline rates of viable eggs/cyst (Trudgill *et al*., 2014). If no host crop is grown, approximately 26 % of PCN population will die per year (Trudgill *et al*., 2014), but if a fully resistant crop is cultivated two thirds of the nematodes will die (Evans, 1993). However, eggs may survive for more than 20 years within cysts and permit populations to readily re-establish if a susceptible host is grown (SASA, 2013). Even if the annual decline of PCN were as high as 33.5%, for *G. pallida* it would take 8 years to maintain population densities (at planting) at a non-damaging limit below 10 eggs/g soil (Trudgill *et al*., 2014).

1.5.3. Chemical control

Nematicides are pesticides that are applied directly to soil in order to limit damage to plants by delaying and reducing the hatching or by killing nematodes. These chemicals can be categorized based on their type of application (fumigant and non-fumigant), active chemical group (e.g. carbamates, organophosphates), and also mode of action (e.g. acetylcholinesterase inhibitor).

Examples of fumigants include halogenated aliphatic hydrocarbons, methyl bromide and 1,3-D methyl isothiocyanate liberators (Franco, 1986; Haydock *et al.,* 2013). These are phytotoxic and must be applied well before planting. Fumigants are very effective and can kill all nematode developmental stages in treated soil including dormant eggs within cysts. For example, 1,3-dichloropropene (1,3-D) kills all nematodes non-selectively and releasesplant nutrients through the mineralization of nitrogen (Evans *et al.,* 2003). It has to be incorporated well before planting the potatoes to reduce particularly high populations of PCN and other soil pests but only in a small area (Minnis *et al*., 2004). The second type of nematicides are non-fumigants. Non-fumigants include organophosphates and carbamates. Their granular or liquid formulations are easily applied. Non-fumigants are less phytotoxic than fumigants, but are toxic to humans and animals. Before using nematicides the soil type and species present must be considered. Non-fumigants are less effective with *G. pallida* which tends to hatch more slowly than *G. rostochiensis* (Evans, 1993). A summary of globally used nematicides is presented in Table 1-1.

The efficacy of nematicides depends on factors including temperature, soil structure as well as microbiological degraders present in the soil (Haydock *et al.,* 2013). An increase in soil temperature may influence the speed of the nematode life cycle as well as changes in microbial and physiochemical degradation of pesticides (Chitwood, 2003). For most nematicides the optimum temperatures are between 7°C and 27°C. Below and above this range, the efficacy of the nematicide might be reduced (Haydock *et al.,* 2013). Nematicides are also subjected to biological and chemical transformations which may influence their toxicity. Use of chemical nematicides is one of the methods of controlling plant parasitic nematodes and so far resistance of field populations to nematicides has not been confirmed and well characterized (Chitwood, 2003). However, because of their effectiveness and large spectrum of activities, nematicides are also non-target biocides and may change flora and fauna in the soil and contaminate groundwater. This could affect nematode competitors, predators and other parasites (Chitwood, 2003). For example long-term aldicarb usage caused decreases in the population levels of rhizobacteria (Sturz and Kimpinski, 1999). These concerns caused the removal from the market of aldicarb and the fumigant 1,3-D since they have not been listed on Annex 1 listing under 91/414/EEC (Hillocks, 2013). According to Clayton *et al*. (2008), currently permitted nematicides in the UK are oxamyl (Vydate 10G), fosthiazate (Nemathorin) and ethoprophos (Mocap 10G).

Table 1-1 Globally important nematicides currently available on world markets (Haydock *et al*, 2013).

Active substance	Chemical group	LD50 (acute oral male	Year of discovery	Example trade name	State of formulation	Manucaturer
Aldicarb	Oxime carbamate	0.93	1965	Temik 10G	Microgranule	Bayer CropScience, www.bayercropscience.com
				Temik 15G	Microgranule	
Carbofuran	Carbamate	8	1965	Furadan 15G	Microgranule	FMC Corporation,
				Furadan 4 F	Liquid	www.fmc.com
Cadusafos	Organophosphorus	37		Rugby 200 CS	Liquid	FMC Corporation,
				Rugby 10G	Microgranule	www.fmc.com
Dazomet	Methyl isothiocyanate	77-220a	1897	Basamid		BASF Corporation, www.agriculturalproducts.basf.com
1,3-Dichloropropene	Halogenated hydrocarbon	150	1956	Telone II	Liquid	Dow AgroSciences,www.dowagro.com
				Telone EC	Liquid	
Ethoprophos	Organophosphorus	62	1966	Mocap 10G	Microgranule	Bayer CropScience, www.bayercropscience.com
				Mocap EC	Liquid	
Fenamiphos	Organophosphorus	6	1967	Nemacur 15G	Microgranule	Bayer CropScience, www.bayercropscience.com
				Nemacur 3	Liquid	
Fosthiazate	Organophosphorus	73	1992	Nemathorin 10G	Microgranule	Syngenta, ww.syngenta.com
Metam (sodium N-methyldithiocarbamate	Methyl isothiocyanate liberator	77-220a	1951	Vapam	Liquid	Amvac Chemical Corporation, ww.amvac-chemical.com
				Vapam HL	Liquid	
Oxamyl	Oxime carbamate	3.1	1974	Vydate 10G	Microgranule	Du Pont, www.1.dupont.com
				Vydate L	Liquid	

1.5.4. Biological control

The potential influence of nematicides on the environment has led to restrictions on their use and resulted in a search for new methods to control PCN. One of the options is biological control. This type of control is less effective on its own and needs much more time than the control normally achieved with nematicides. Successful application of biocontrol agents depends on integration with other control methods such as use of resistant cultivars, trap cropping and crop rotation (Kerry, 1997, Tobin *et al.,* 2008). Biological control is mostly based on interactions between predator nematodes, fungi, bacteria and mycoplasma-like organisms and PCN (Ferris *et al*., 1992; Khan and Kim, 2007) (Figure 1.5). Some fungi produce structures that are traps for mobile stages of PCN, the J2s and males. The most well-known species of trapping fungi is *Arthrobotrys oligospora*. Other fungal biocontrol agents such as *Pochonia chlamydosporia, Paecilomyces lilacinus, Cylindrocarpon destructans* and *Plectosphaerella cucumerina* attack eggs and females of PCN. One of the most promising of these, *Pochonia chlamydosporia,* has been used as a model organism for the study of methods of culture and incorporation in soil (Evans, 1993)**.** When an inoculum of straw colonized by *C. destructans* was placed around potato seed tubers planted in PCN infested soil in pots kept in a glasshouse, the numbers of juveniles of *G. rostochiensis* and *G. pallida* in roots were decreased by 62% and 84%, respectively (Evans, 1993)**.** *Pochonia chlamydosporia* and *P. lilacinus* have also been studied and the possibility of using them as biological control agents considered (Tobin *et al.,* 2008). Endoparasitic fungal organisms such as *Drechmeria coniospora*, *Hirsutella rhossiliensis* and *Verticillium balanoides* with

characteristic adhesive spores which attach to the nematode cuticle and attack infective nematodes in soil (Kerry, 1997; Kerry *et al*., 2002) have also been studied as potential biocontrol agents.

Some examples of bacteria that have been considered as biocontrol agents for PCN are *Agrobacterium radiobacter, Bacillus subtilis* and *Pseudomonas* spp. The mode of action of these bacteria differ, and include direct effects on hatching and nematode motility, and indirect effects such as modifications of root exudates (Kerry *et al*., 2002).

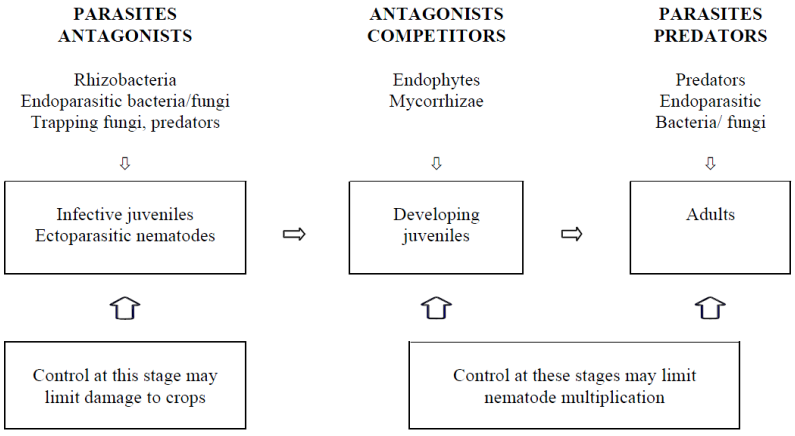


Figure 1.5 Pathways of relations between natural enemies of PCN and possible impacts on control (from Kerry *et al*., 2002).

1.5.5. Trap cropping

The main purpose of using cultivars for trap cropping is to stimulate hatching of juveniles from eggs and prevent their reproduction resulting in a decrease in the populations of parasites in infested fields. The multiplication of PCN can be prevented by using resistant cultivars or by the destruction of the plants before adult females are fertilized so that no new generation of PCN can develop (Whitehead and Turner, 1998; Scholte, 2000; Timmermans, 2005;). The advantage of control by trap cropping is the potential reduction of both species of PCN by 75% to 87% (La Mondia and Brodie, 1986; Halford, *et al*., 1999). However, timing plays a crucial role and must be precise otherwise instead of a reduction, the PCN population will increase. One of the most attractive methods is using non-tuber-forming members from the *Solanum* family with resistance to PCN which have a stimulatory hatching effect on PCN juveniles, and good plant performance under temperate climatic conditions (Scholte, 2000). *Solanum sisymbriifolium* is an example of a species that triggers hatching and in which PCN is not able to complete its life cycle. *S. sisymbriifolium* is a non host of PCN, easy to destroy because it does not produce tubers and may reduce population of PCN by 75%. Nevertheless, it is slow to establish in cooler locations and requires fertilization and weed control (Kerry *et al*., 2002).

1.6. Modelling population dynamics and damage levels

1.6.1. Population dynamics

Most of models describing population dynamics of PCN are based on the main variables: nematode, host and environmental factors. An understanding of the

mechanisms that are involved in these interactions is needed in order to predict population dynamics and yield losses from the initial population densities of PCN (Pi) (Trudgill and Phillips, 1997).

One of the first models predicting population changes of PCN was written by Seinhorst (1967). The proposed model was for sedentary nematodes with one generation, such as PCN. The equation presents the relationship between the densities of initial (Pi) and final (Pf) populations.

$$Pf = a\,(-\ln q)^{-1}\,(1-q) = 1-q^{Pi}\,/-\ln q$$

where *a* is the maximum rate of multiplication and *1-q* is the proportion of the available area which is exploited for food at a density of *Pi=1* (Seinhorst, 1967). The relationship between these the Pi and Pf is non-linear. The model predicts that the maximum rates of multiplication are at low initial densities. As *Pi* increases the rate of multiplication is reduced, and in reality at high *Pi's* root damage can be sufficient so that the population growth becomes negative and the final population size is reduced relative to the initial population. The second model proposed by Jones and Kempton (1978) is based on a logistic equation and includes information on the sex ratio which is density dependent (Trudgill, 1967). The described equation is presented as:

$$P_f = \frac{a(1-Cp)Pi}{1+(a-1)P_i/c(\frac{E}{c})^{Pi/E'}} + CpP_i$$

where P_f is expressed as a proportion of the logistic equation, *a* represents a measure of female development and fecundity, *Cp* is the amount of unhatched nematodes, *E'* is the actual equilibrium density in eggs/g soil, *E* is proportional equilibrium density and c is equivalent to *z-T* from Seinhorst (1965) (McSorley and Phillips, 1993).

Phillips *et al*. (1991) modified the above model by adding a variable for resistance and included a parameter which describes the sex ratio. The equation takes the form:

$$P_f = \frac{f^{'(1-Cp)} Pi}{1 + b^{'}(1 - Cp)P_i(1 + P_i/c)} + CpP_i$$

where *f'* is related to female fecundity and *b'* to sex ratio.

Ward *et al*. (1985) constructed a dynamic model simulating the population dynamics of *G. pallida*, and its effect on potato development by combining two sub-models: the PCN population model and a potato growth model. Another dynamic model has been written by Moxnes and Hausken (2007). The basic equation of this model is focused on the number of eggs per unit mass of soil at time *(t)*:

$$N(t) = L(t) - H(t) + R(t)$$

where *L(t)* is rate of loss of eggs per time, *H(t)* is the hatching rate of eggs per time and *R(t)* value of new eggs (reinforcement). Their model includes also age

dependency, simulated impact of changes of the season length, planting resistant potatoes and nematicides.

The models presented above can be used to clarify the optimal strategy to control or reduce population density of potato cyst nematodes with a particular set of conditions.

1.6.2. Damage levels

Prediction of damage levels and yield loss is necessary to estimate losses caused by PCN. Seinhorst in 1965 described the relationship between nematode density and damage to plants and made the assumption that as the population density (*Pi*) rises a tolerance limit (*T*) is reached where yield is reduced. The third parameter in the Seinhorst equation is *z*, a constant slightly less than one. The equation is:

$$y = m + (1 - m)z^{(Pi-T)}$$

if Pi>T and y=1 if Pi≤T

where y is the yield and is expressed as a proportion of the nematode-free yield and m presents the ratio of minimum (*Pi*=∞) to maximum (*Pi=0*) initial population. According to Seinhorst (1965), the bigger the yield potential, the greater the loss in tonnes per hectare (Trudgill and Phillips, 1997).

Oostenbrink (1968) suggested that relationship between yield loss and PCN population density is linear. The equation for such a line is:

y = y(max) - slope constant × log Pi

Even though this equation is simpler than the yield loss relationship described by Seinhorst (1965), it is still expressed in proportional rather than actual (tonnes per hectare) terms. Also, without experimentation it is impossible to establish the slope of the regression. However, even after including additional information these models are still purely descriptive and do not predict yield losses in real situations. To simplify them Elston *et al*. (1991) introduced an equation based on data from field trials that is an inverse linear model:

$$E(Y) = Ymax/[1 + \left(\frac{Pi}{c}\right)]$$

where *Ymax* presents yield expected from a non-infested yield, *T* is a threshold for *Pi* below which no damage occurs and c is the rate at which increasing *Pi* decreases expected tuber yield. Phillips *et al*. (1998) showed that is possible to improve the model by splitting the *c* parameter into two components: the first describing the contribution from the genotype and cultivar tolerance differences (*g*) and the second (*s*) a site component. After transformation the equation takes the form:

$$E(Y) = Ymax/[1 + \left(\frac{Pi}{g * s}\right)]$$

The above models have been developed to provide a basis for predicting the outcome of different control strategies that might be employed in an integrated pest management programme for sustainable potato production.

1.6.3. Integrated control of potato cyst nematode

The control of PCN is likely to be much more effective when methods are combined in integrated control programmes. These include selective use of resistant cultivars that permit relatively little net multiplication, the use of trap crops and biological control agents (Evans, 1993). It may be necessary to alternate resistant and susceptible crops to prevent the selection of virulent nematodes on resistant cultivars from avirulent populations (Minnis *et al*., 2002; Turner and Fleming, 2002). With the loss of nematicides due to changes in EU legislation and the increasing distribution of *G. pallida* for which there are few cultivars available with high levels of resistance, tools such as population dynamic models are becoming more and more important.

An understanding of the mechanisms that are involved in interactions between environment, host and presence of mixed populations in the field and nematodes is needed in order to predict population dynamics and yield losses (Trudgill and Phillips, 1997). The current version of the Potato Council's PCN Model was written as part of Sustainable Arable Link Programme LK0918 (CSA5701A) "Integrated Management Strategies for PCN" in 2005 as a collaboration between Scottish Crop Research Institute (now The James Hutton Institute), Rothamsted Research and Harper Adams University College (Haydock, 2010). The CD-based version of The Model has been replaced by an online PCN Calculator; Integrated Control of *Globodera pallida* (AHDB, 2009). The PCN PCL model has been developed to provide a basis for predicting the outcome of different control strategies that might be employed in an integrated pest management programme for sustainable potato production. An example of

the estimates and predictions generated by the online PCN calculator is shown in Figure 1.6. Currently this calculator is used as an educational tool and does not offer advice on what grower should do; however, it may play a role in decision support. It shows the grower the implications of the applied control methods on *G. pallida* infestation and the predicted yield. Growers with access to The Model or online PCN calculator can test the effect of various variables (potato variety, soil type, initial population and nematicide) on long term population changes and ware yields.

However, the PCN calculator does not predict the effect of temperature on PCN population dynamics or what happens when mixtures of both species are present in the same field. Temperatures are expected to continue to increase due to climate change which has implications for soil temperatures and PCN population dynamics. Therefore there is a need to assess the impact of temperature on PCN by adding parameters that describe this important environmental factor.

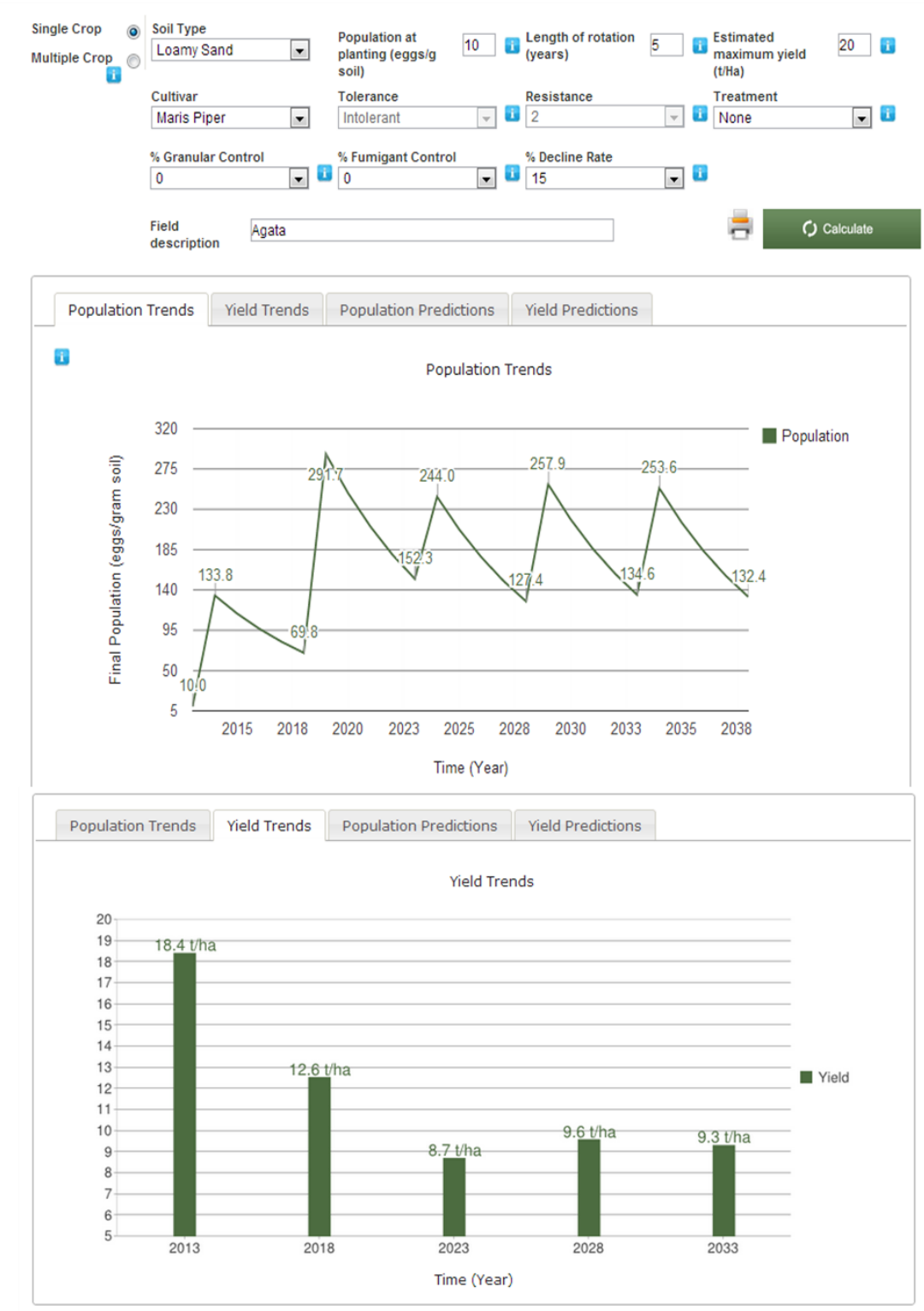


Figure 1.6 An example of predictions from PCN calculator with cv Maris Piper in a 5 year rotation over 20 years without applied nematicides and a 15% population decline rate.

1.7. Aims and Objectives

The main purpose of this study was to investigate aspects that were lacking in the PCN PCL model particularly relating to the impact of temperature on PCN population dynamics and the damage caused by them. The goal was to produce parameters that would describe the impact of different soil temperatures that are relevant to current situations in the UK and in terms of future climate change.

The specific objectives were:

1. Investigate the differences in hatching i.e. time delay, preferred temperatures and length of hatching between two species in terms of temperature *in vitro*.

2. Investigate the influence of temperature on nematode development on host plants, including the adult stages, in growth cabinets with temperatures corresponding to field conditions.

3. Examine the potential for a second generation of PCN.

4. Determine the duration of the potato cyst nematodes' life cycle and the number of generations per year in different initial population densities, temperature regimes and agroecological conditions.

5. Investigate the interaction between two species of PCN in mixed populations.

6. Characterize molecular variation within populations of potato cyst nematode *G. pallida* collected from different regions in the UK and compare them with those from other European countries.

2. TEMPERATURE EFFECTS ON DEVELOPMENT AND REPRODUCTION OF PCN

2.1. Introduction

Damage caused by PCN is affected by a range of factors including environmental conditions, soil type, potato cultivar, fertilization and other pathogens. According to several reports (Franco, 1979; Schmidt *et al.,* 1993; Trudgill *et al.,* 2005; Kakaire *et al*., 2012; Van der Waals *et al*., 2013), one the most influential environmental factors affecting nematode development is temperature. Nematodes, like all ectothermic animals, depend on external heat sources to maintain their body temperature. They are also poikilothermic organisms whose body temperature is almost identical to that of their environment. During their life cycle, different nematode activities have specific temperature requirements (Franco, 1979). For example, temperature plays a very important role in the “rate of embryogenesis and post infection development of polikothermic nematodes” (Koenning and Sipes, 2000). Many nematodes are adapted to particular temperature ranges and have different optimum temperatures for feeding, hatching, reproduction and survival (Neilson and Boag, 1996). For *Meloidogyne arenaria* eggs in early stages of development, the optimal temperature is ~15°C (Ferris *et al.,* 1978). According to Ambrogioni *et al.* (2000) the most suitable soil temperature for reproduction and development of *Globodera tabacum* in Italy is 26°C. The basal threshold temperature for this nematode was estimated to be 11°C and at 32°C their invasion of the host was inhibited. Strajnar *et al*. (2011) suggested that in order

for *M. ethiopica* to complete its reproduction cycle, daily temperatures of 18-26°C are required, but at 13°C and lower it is not able to reproduce.

It is reported that *G. pallida* populations hatch and reproduce at lower temperatures than *G. rostochiensis* populations, however *G. rostochiensis* is more successful than *G. pallida* at temperatures above 20°C (Franco, 1979). In northern Europe, there is usually one main generation of potato cyst nematodes per year (Jones, 1950), although there are several studies which describe the occurrence of a partial second generation. Jones (1950) observed that suitable soil temperatures may permit more than one generation of *Heterodera* (later reclassified as *Globodera*) per year. Evans (1969) has also observed with *Heterodera rostochiensis,* a slight increase in the number of juvenile nematodes in the roots during August in Long Island, U.S.A., which might have indicated a small second generation. Greco *et al*. (1988) recorded a completed second generation of *G. rostochiensis* at Avezzano in Italy and Jimenéz-Pérez (2009) observed a second generation of *G. rostochiensis* at soil temperatures of 18°C in Venezuela and a lack of entry into diapause; PCN is thus capable of greater multiplication giving greater challenges in controlling population levels, if the second generation is not complete it might be an opportunity to decrase the population level by harvesting in right time. The occurrence of a second generation may be related with PCN’s ability to adapt to new environmental conditions and to evade the diapause stage.

2.2. Aims and Objectives

The principal objective of this chapter was to investigate the relationship between temperature and the life cycle of both species of PCN.

The specific objectives were:

1. Investigate the differences in hatching i.e. time delay, preferred temperatures and length of hatching between two species in terms of temperature *in vitro*.

2. Investigate the influence of temperature on nematode development on host plants, including the adult stages, in growth cabinets with temperatures corresponding to field conditions.

3. Examine the potential for a second generation of PCN.

2.3. Materials and Methods

2.3.1. Effect of different temperatures on the hatching of *G. rostochiensis* and *G. pallida* populations in root diffusate and water

2.3.1.1. Nematodes

Cysts from *G. rostochiensis* population A (pathotype Ro1) and *G. pallida* population E/Lindley (pathotype Pa2/3) were obtained from The James Hutton Institute PCN collection and were stored at 4°C for at least one year. For the experiment, cysts were randomly selected and had previously been sieved to 250 µm to exclude small or damaged cysts.

2.3.1.2. Potato Root Diffusate

The *Solanum tuberosum* cv Desirée was used for producing potato root diffusate (PRD). Sprouts were planted about two weeks before the experiment was started. After two weeks, the aerial part of the plant was discarded, soil was removed from the roots and the roots were washed in water. Then the roots were placed in 250 ml of sterile distilled water for at least 4 hours. The liquid was passed through Whatman filter paper No 1 and stored at 4°C (Rawsthorne and Brodie, 1986).

2.3.1.3. Hatching test using a temperature gradient table

Experiments were performed on a thermal gradient table (Grant GRD 1 Camlab, Cambridge, UK) that generated a temperature gradient across a metal plate. The table was set to have a continuous temperature range of 5°C to 29°C with 11 positions across the gradient defined by a plastic grid. Ten cysts were

exposed to 2 ml of hatching agent (PRD) or sterile distilled water (SDW) in a 5 cm Petri dish with 5 replicates (PRD) and 2 replicates (SDW) per temperature (Figure 2.1). PRD and SDW was refreshed 8 times during the experiment, at three–four day intervals, each time juveniles were collected and counted. To determine whether daily fluctuations of temperature influenced the hatching behavior of PCN, a second experiment was performed on a temperature gradient table (Grant GRD 1 Camlab, Cambridge, UK) where the temperature was altered every 12 hours) (Table 2-1).

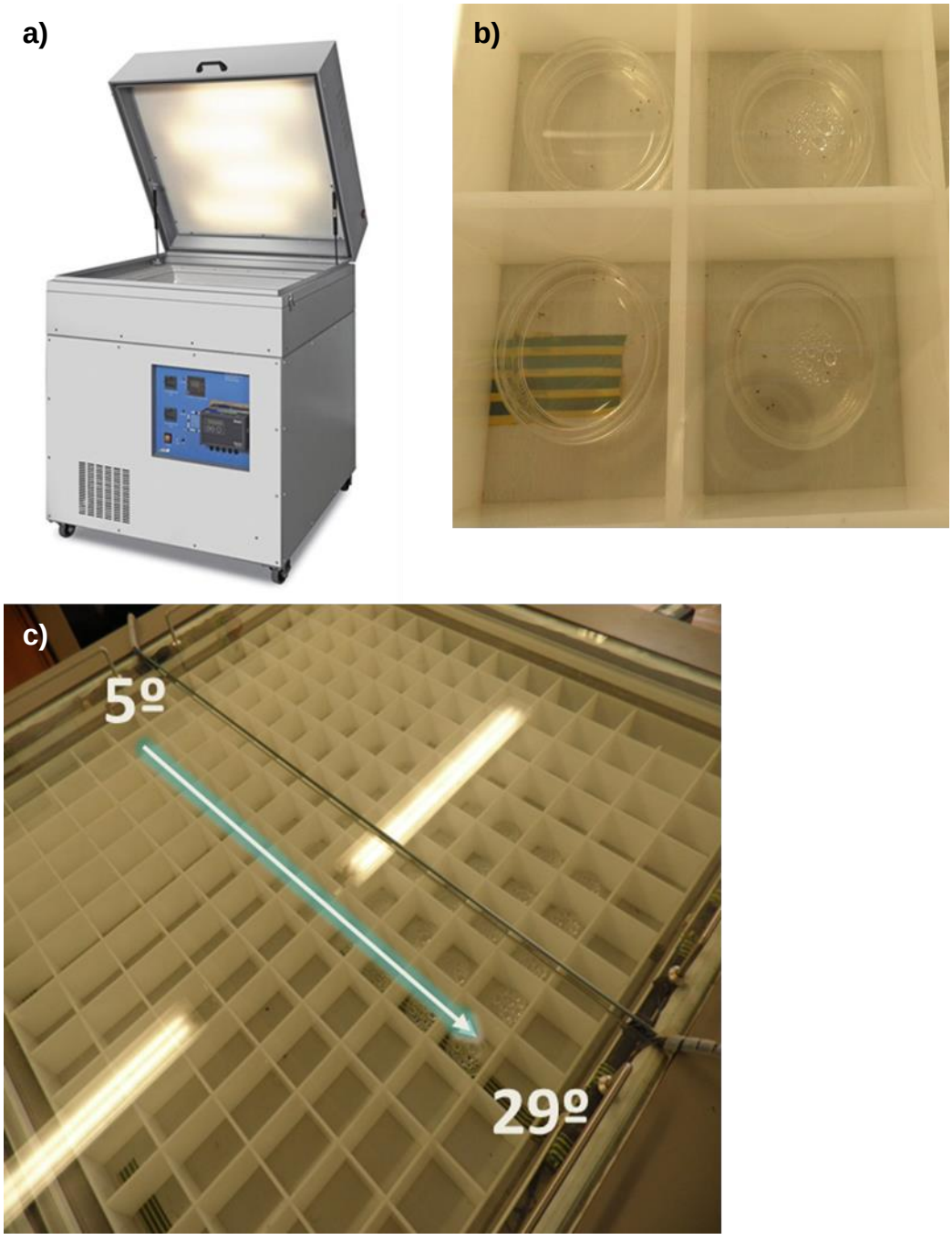


Figure 2.1 Thermal gradient table (Grant GRD 1) (a) with a selection of the Petri dishes containing cysts of PCN in PRD or H_2O (b) arranged within the plastic grid on the thermal gradient table (c).

Table 2-1 Average temperatures at the positions of Petri dishes (marked as blue squares) containing cysts on the thermal gradient table. Temperatures on the diagonal from bottom left to top right were constant and those at all other position fluctuated with the maximum temperature change of 10°C at the top left and bottom right positions.

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

The accumulated number of hatched J2 was determined by removing the liquid from each Petri dish every 3-4 days into a 12 well multiwell plate and counting the juvenile nematodes using a stereo-microscope (Olympus S7-ST). To determine the number of unhatched eggs at the end of the experiment, the cysts were collected from the Petri dishes and crushed in a glass homogenizer (GPE Scientific, 20104), and unhatched eggs and juveniles were counted. The

total unhatched and hatched number of nematodes was used to determine the percentage of hatch.

2.3.2. Development of females of *G. pallida* and *G. rostochiensis* on roots of different potato genotypes

2.3.2.1. Nematodes

The *G. rostochiensis* population A (pathotype Ro1) from 2010 and *G. pallida* population E/Lindley (pathotype Pa2/3) 2009 used in these experiments were taken from The James Hutton Institute PCN collection and selected as described in 2.3.1.1. Cysts were homogenized with a Citenco Homogenizer (Jencons Scientific Ltd) in 250 ml of sterile distilled water and the inoculum density adjusted to 300 eggs/ml. For the estimation of egg concentration three counts were made.

2.3.2.2. Plant material

The *Solanum tuberosum* cultivars used in these experiments were the susceptible cv Desirée, cv Vales Everest (partially resistant to *G. pallida*) and cv Maris Piper (fully resistant to *G. rostochiensis*). Small tubers or tuber pieces were placed in plastic transparent canisters containing 200 ml of general purpose compost mixture (Figure 2.2) (Foot 1977; Phillips *et al*., 1980). Each canister was inoculated with 10 ml of inoculum (3000 eggs/canister). Canisters were arranged in rows and randomized within each row (Table 2-2) with 3

replicates of each cultivar per temperature. The middle column had canisters only containing soil and a temperature probe to monitor temperatures.

Figure 2.2 Example of canister containing compost, potato tuber piece, daughter tubers and cysts of either *G. pallida* or *G. rostochiensis* which was used to test for female emergence at different temperatures.

3.2.2.3. Emergence of PCN females over a temperature gradient

Each week canisters were removed from the gradient table and female nematodes on the surface of the roots were recorded by observing the roots at the side of the canister with an illuminated magnifying lens.

Table 2-2 Randomisation plan for the three replicates of the canisters for the *G. pallida* female emergence experiment with the susceptible cv Desirée (Des) and the partially resistant cv Vales Everest (V. Everest) on the temperature gradient table. The middle row had canisters with only soil and a temperature probe.

Des 1	V. Everest 3	V. Everest 2	22 ^{0} C	Des 2	V. Everest 1	Des 3
V. Everest 1	Des 2	V. Everest 3	20 ^{0} C	Des 1	Des 3	V. Everest 2
V. Everest 3	Des 2	Des 1	18 ^{0} C	V. Everest 1	Des 3	V. Everest 2
Des 1	V. Everest 1	V. Everest 3	16 ^{0} C	Des 2	V. Everest 2	Des 3
V. Everest 1	V. Everest 2	Des 2	14 ^{0} C	V. Everest 3	Des 1	Des 3
V. Everest 3	Des 3	V. Everest 1	12 ^{0} C	Des 2	V. Everest 2	Des 1
Des 1	V. Everest 2	V. Everest 1	10 ^{0} C	Des 3	Des 2	V. Everest 3

2.3.3. Growth cabinet experiments

2.3.3.1. Nematodes

The cysts for *G. rostochiensis* population A (pathotype Ro1) and *G. pallida* population E/Lindley (pathotype Pa2/3) used in these experiments were taken from The James Hutton Institute PCN collection and selected as described in 2.3.1.1. Selected cysts were packed into nylon bags with 30 cysts/bag.

2.3.3.2. Plant material

The *S. tuberosum* cultivars used for these experiments were the susceptible Desirée and the cv Morag which has partial *G.pallida* resistance derived from S*olanum vernei*. Plants were grown in pots containing 500 gm sand:loam (50:50) which had previously been autoclaved and mixed. A single potato sprout on a spherical piece of tuber cut from a seed tuber was planted in each pot. Plants were grown in the greenhouse until they were ~10 cm high before being moved to three (*G. rostochiensis* experiment) and four (*G. pallida* experiment) growth cabinets (Phytotrons) (SANYO, Model 1700). The plants were arranged in a randomized design and two days later a cyst bag was planted into the soil of each pot beside the tuber piece (Figure 2.3). Five weeks after inoculation the cyst bags were removed from the pots.

2.3.3.3. Growth cabinet conditions

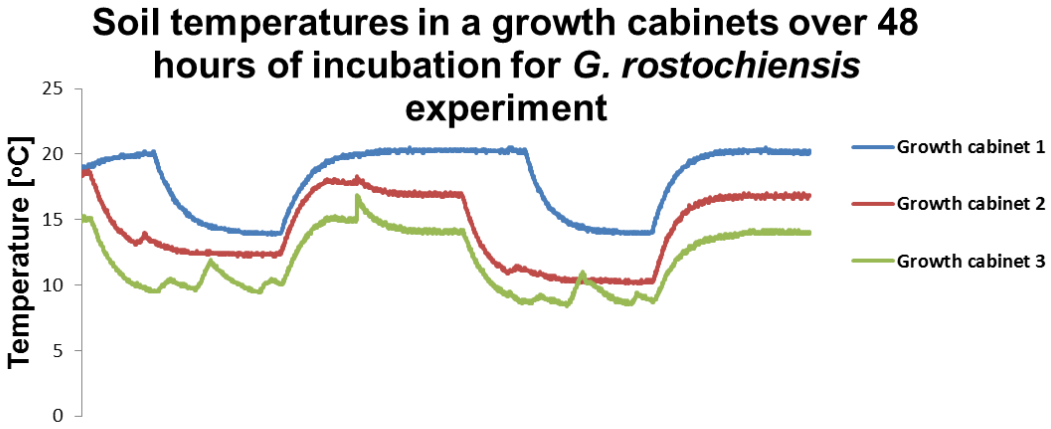
Experiments were performed in Phytotrons in which conditions were set to achieve average day time soil temperatures of 11°C, 14°C, and 17°C or 18°C for 16 hours with light, with night-time temperatures 5°C lower for each temperature respectively (Figure 2.5). Plants were watered on a daily basis and fed weekly. The relative humidity inside the growth cabinets was maintained at 75%. Temperatures in the pots were monitored with DS1920-F5 Temperature ibuttons (HomeChip, Milton Keynes, UK).

2.3.3.4. Extraction of nematodes

Hatched juveniles and adult free living males from three pots per temperature (*G. rostochiensis*) or two pots per temperature and cultivar (*G. pallida*) were extracted with a Baermann funnel (Viglierchio and Schmitt, 1983) at 7–10 day intervals over a fifteen week period (Figure 2.4). Additionally, in the *G. pallida* experiment, the total number of cysts recovered from the sieves per pot was scored. In order to assess the number of eggs per cyst, cysts were homogenized by the Wheaton Dounce Glass Tissue Grinder Homogenizer and three counts/ ten cysts were made.

Figure 2.3 Inoculated potato plants in a growth cabinet (18°C) with Desirée plants inoculated with bags of 30 cysts each of *G. rostochiensis*. Cyst bags can be seen protruding from the soil.

Figure 2.4 Baermann funnel used for collection of juveniles and male nematodes.



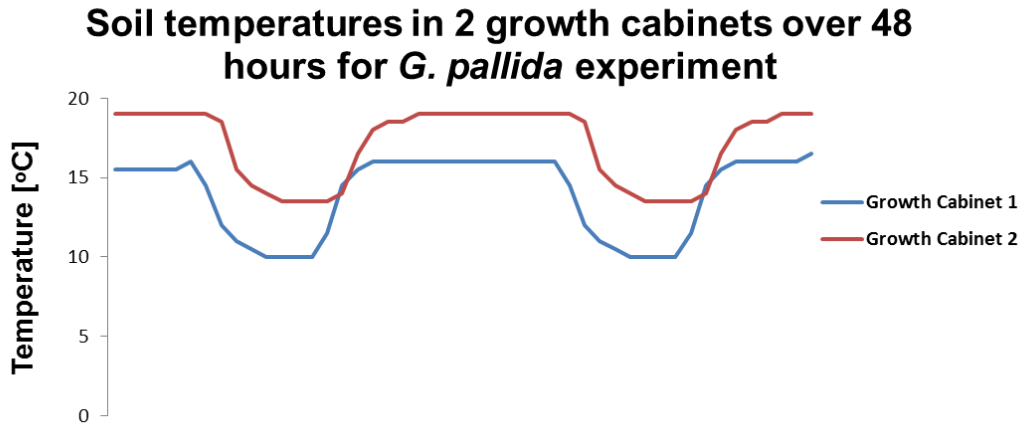


Figure 2.5 Soil temperatures inside the pots for 2 (*G. pallida)* and 3 (*G. rostochiensis)* growth cabinets over 48 hours of incubation.

2.3.2.5. Cyst sizing

To determine the effect of partial resistance on cyst development and fecundity, cyst sizing was performed. The size and volume of newly formed cysts were established by taking pictures of the collected cysts (Microscope Olympus SZ-CTV and Camera Micropublisher 3.3RTV) and measuring the pixel area on the pictures by using software developed by Sebastian Eves-van den Akker (script attached to appendix 1). The mean diameter of 10 randomly selected cysts was established and volume of each cyst estimated.

2.3.4. Statistical analysis

The results obtained from the experiments were transformed and analysed using GenStat Version 14.1 and Microsoft Excel Version 14.0.4760.1000. For the hatching rate experiments, logistic curves were fitted to the cumulative proportions of eggs hatched and analysis of variance was carried out on the parameters of the curves to test for differences in the hatching behaviour between the two species of potato cyst nematode, at different temperatures and in PRD or H_2O. Tests for interactions between species and temperature were included.

The data (numbers of J2, males, females and eggs) were analysed using repeated measurements of variance and standard analyses of variance.

Depending on the distributions of residuals, the data were subjected to logarithmic or square root transformations to normalise the variances.

2.4. Results

2.4.1 Effect of different temperatures on the hatching of *G. rostochiensis* and *G. pallida* populations in potato root diffusate and H_2O

2.4.1.1 Hatching at constant temperatures

The first set of experiments examined the influence of temperature on hatching of PCN at constant temperatures from 5–29°C. To determine the amount of spontaneous hatch in H_2O, 10 cysts of each species were incubated in water for 35 days. The percentage of total hatch was <4% (Figure 2.6). There was an overall significant response of PCN to temperature when hatching in water (P=0.018). However, no significant differences in relation to spontaneous hatch and temperature and species (P=0.373) were recorded.

The differential effect of temperature on the final proportions of eggs that hatched in PRD for both species of PCN is shown in Figure 2.7. Estimating the proportion of hatched nematodes in PRD for the temperature range of 5–9°C for *G. rostochiensis* was not possible due to the loss of these samples. The cumulative hatch for *G. rostochiensis* and *G. pallida* is shown in Figure 2.8. Both species showed a delayed hatch after 20 and 30 days respectively (Figure 2.8 and Figure 2.9). For *G. pallida* there was no significant difference in the total

hatching from 13–25°C. The lowest cumulative hatch for *G. pallida* was recorded at 29°C followed by 5°C and 7°C, whereas for *G. rostochiensis* the lowest cumulative hatch was at 5°C and 7°C.

The hatching curves (Figure 2.8 and Figure 2.9) show that most hatching occurred within the first 3 weeks of incubation. *G. rostochiensis* hatched more rapidly than *G. pallida* with the first emergence of juveniles observed after 3 days at temperatures between 17–27°C whereas the first occurrence of *G. pallida* juveniles was recorded after 5 days of incubation at temperatures between 17–23°C. For *G. rostochiensis* a rapid increase in hatching was recorded on day 7 and hatching declined after 11 days whereas for *G. pallida* hatching continued to increase until day 17 and then subsequently declined.

A repeated measurements ANOVA was applied with temperature and time as factors to compare the impact of temperature on hatching of both species. For both species the comparison of the percentage hatch induced by PRD revealed significant differences ($P<0.001$) in hatch stimulation at different temperatures. The highest hatch of second stage juveniles (J2) occurred for both species in intermediate temperatures. *G. rostochiensis* hatched faster than *G. pallida*; however, *G. pallida* showed better adaptation to lower temperatures. The differences in hatching times due to varying temperatures were also significant ($P<0.001$).

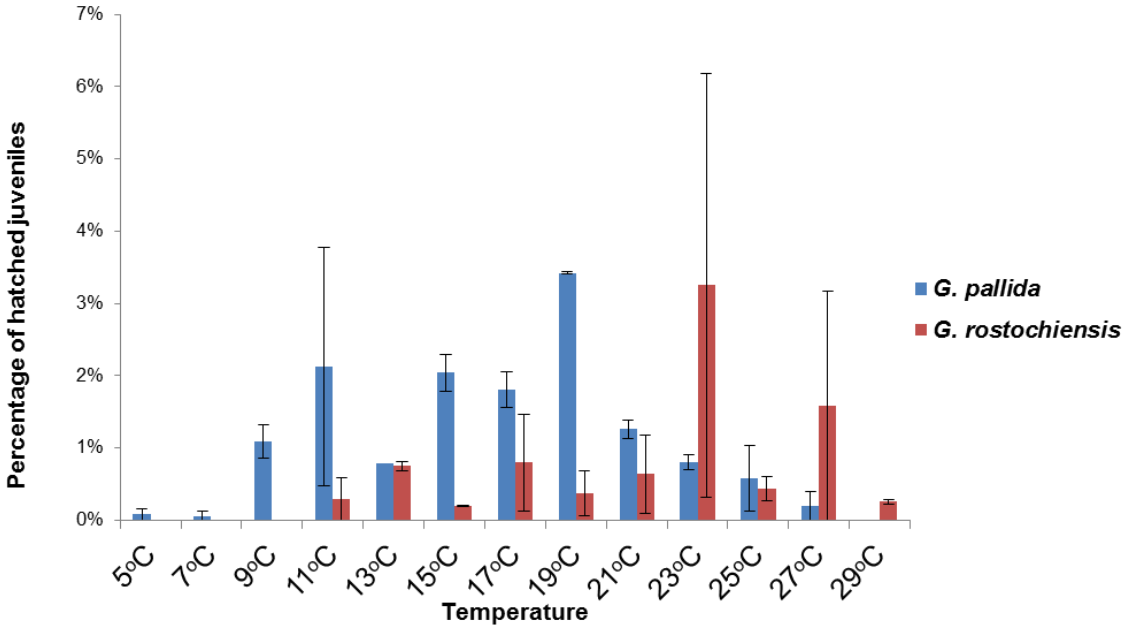


Figure 2.6 Proportion of total hatch of *Globodera rostochiensis* and *G. pallida* in SDW at constant temperatures from 5–29°C after 35 days of incubation.

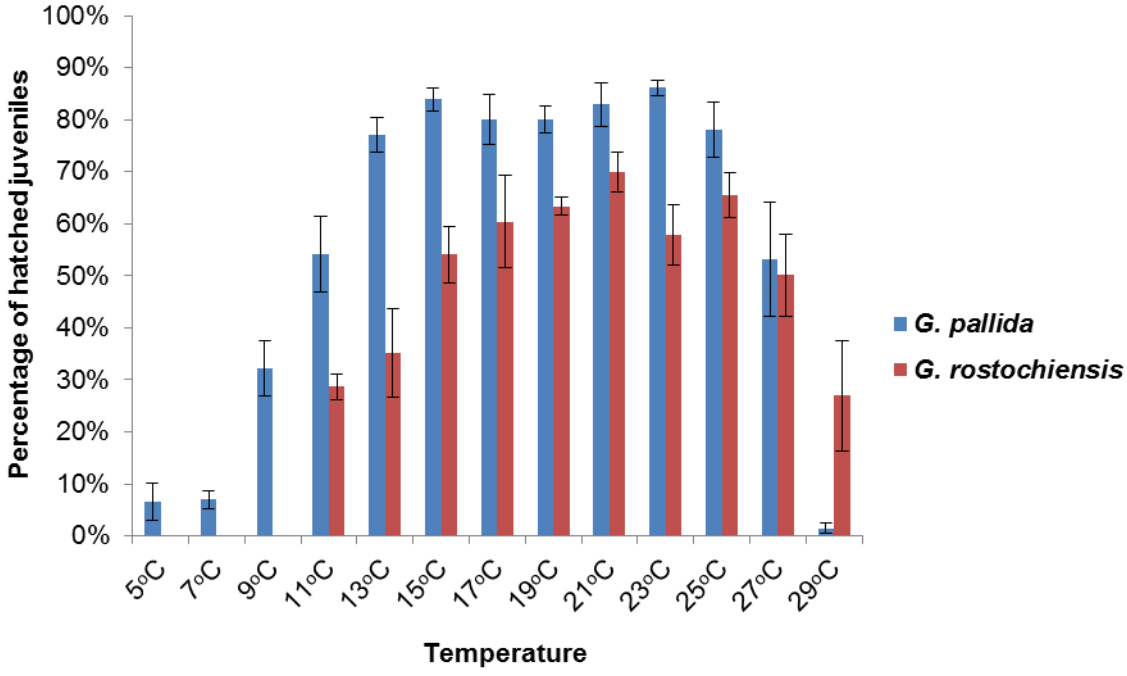


Figure 2.7 Effect of different constant temperatures (5–29°C) on total percentage of hatching of *Globodera rostochiensis* and *G. pallida* in potato root diffusate (PRD) after 35 days incubation.

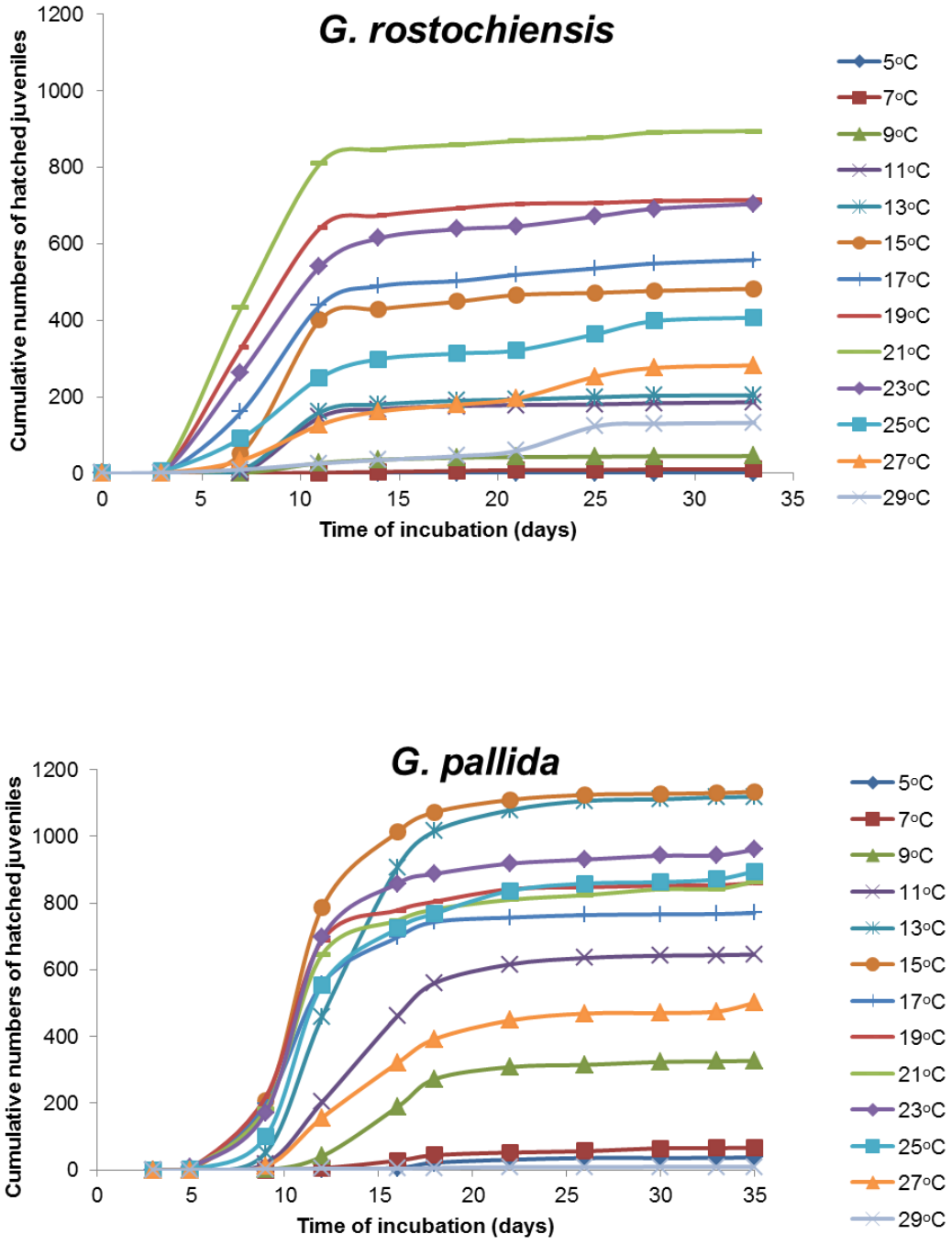
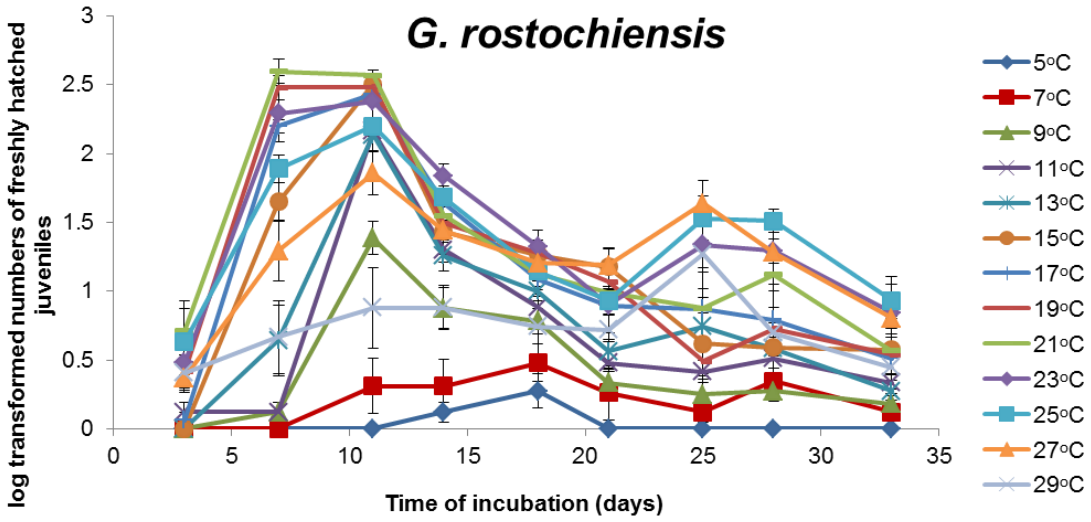


Figure 2.8 Cumulative numbers of hatched juveniles (J2) from cysts of *G. rostochiensis* and *G. pallida* in potato root diffusate (PRD) over 35 days of the incubation at constant temperatures from 5–29°C. Data are expressed as the accumulation of juveniles that have hatched at a particular temperature and are means of 5 replicates.



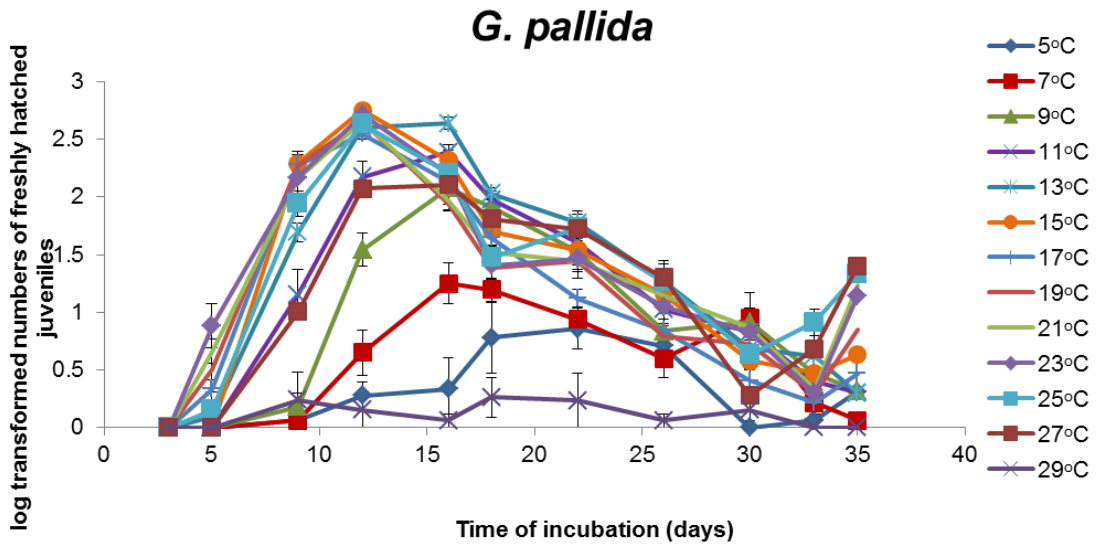


Figure 2.9 Numbers of freshly hatched juveniles of *G. rostochiensis* and *G. pallida* over 35 days of incubation at constant temperatures from 5–29°C. Vertical lines indicate standard errors of the means of the 5 replicates.

2.4.1.2 Hatching test with diurnal temperature fluctuations

In order to establish the impact of a diurnal temperature fluctuation on the hatching of PCN, a temperature gradient table was programmed to produce a diurnal temperature cycle. Every twelve hours the temperature gradient switched (Table 2-2) and resulted in a spectrum of temperature ranges from constant to a maximum of 10°C change between 8.4–17°C. As observed previously, *G. pallida* showed a greater overall hatch. Both species favoured higher temperatures; however *G. pallida* showed higher hatching in lower temperatures. Results indicate also that hatching in constant and fluctuating temperatures are not significantly different for both species of PCN. The percentage of total hatched nematodes for both PCN species is shown in Figure 2.10 at the mean daily temperature. Due to the design of the gradient table, some temperature combinations had only one replicate therefore estimating the variability for these was not possible.

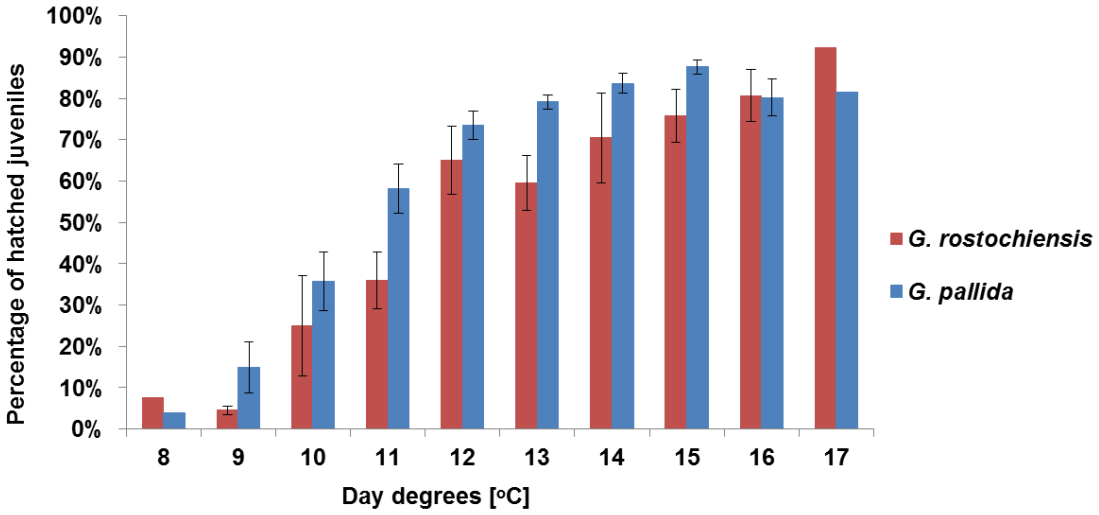


Figure 2.10 The total hatch of *Globodera rostochiensis* and *G. pallida* in potato root diffusate (PRD) against average temperature (°C) using the diurnal temperature gradient table (for temperatures see Table 2-1). Data are expressed as percentages of the maximum hatch per mean of daily temperatures and standard errors are shown for replicated samples.

2.4.1.3 Logistic nonlinear regression model

As shown in Figure 2.10, the curve describing the cumulative hatching of PCN nematodes over a range of temperatures has a hyperbolic shape. The cumulative proportions of hatched nematodes were used to estimate the parameters of a logistic curve describing the hatching rates for each plate.

$$Y=A + C/(1 + EXP(-B*(X - M)))$$

where Y is the number of hatched nematodes, A is the lower asymptote, an estimate of the number hatched at time zero, C the maximum asymptote, an estimate of the proportion expected to hatch at any given temperature, A+C is the upper asymptote, B is the slope of the intermediate portion of the curve, M

is the point of inflection which gives an estimate of the number of days until half of the eggs that are going to hatch have hatched. The fitted curves from the logistic model were used to calculate values and standard errors for the maximum rate of hatching. The total numbers of eggs hatched in different temperatures, estimated by the sum of the two asymptotes (A and C), was used for estimating the parameters.

2.4.1.3.1 Comparison of parameters from the hatching tests with constant temperatures

The parameters of a logistic curve were estimated from the data. The parameters B, C and M of the logistic curve varied according to the mean temperature (Figure 2.11). The parameter M indicated that *G. pallida* requires a longer time of incubation for half the final amount of hatching to occur than *G. rostochiensis*. ANOVA confirmed that for M there were significant differences in the mean response at different temperatures, and for the 2 species, as well as an interaction between temperature and species ($P<0.001$). For parameter C, the ANOVA revealed that there were significant differences in the mean response at different temperatures ($P<0.001$). *G. pallida* had a significantly higher response than *G. rostochiensis* in the amount of hatched nematodes ($P=0.005$), however no interaction between temperature and species was noted ($P=0.063$). ANOVA on the B values showed significant differences between the mean response at different temperatures ($P<0.001$), with no difference between the two species ($P=0.449$) but a significant interaction between species and temperature ($P<0.001$). *G. rostochiensis* had a higher response at lower

temperatures than *G. pallida* resulting in quicker hatching, however at temperatures above 21°C *G. pallida* hatched faster.

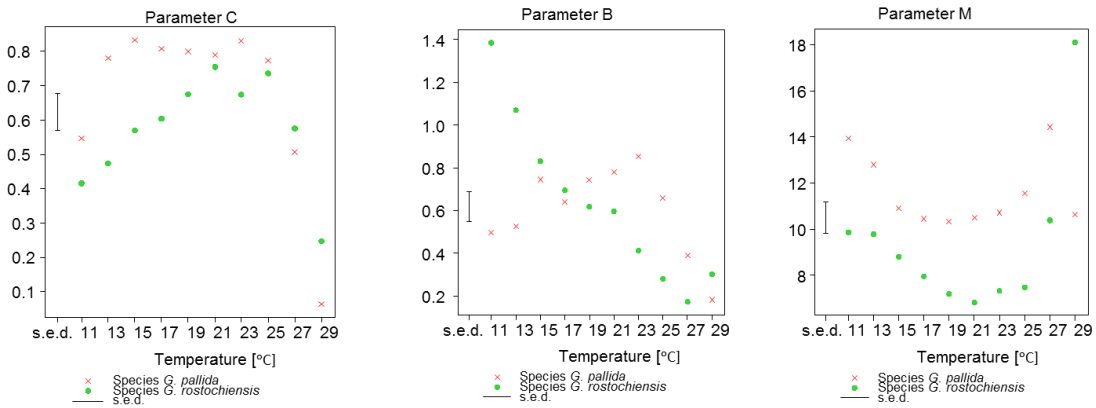


Figure 2.11 Comparison of the variation of the parameters C, B and M in the logistic model in terms of temperature in the constant temperature regime experiment for both PCN species (*Globodera pallida* (red) and *G. rostochiensis* (green)).

2.4.1.3.2 Comparison of parameters from the hatching tests with diurnal temperatures

The main goal of these experiments was to investigate to determine if hatching differs in constant versus comparable average fluctuating temperatures. It was previously shown that the total percentage hatching of PCN over a range of temperatures has a hyperbolic shape and that the parameters A, B, C and M of the logistic curve vary according to the mean temperature. Boxplots of the curve parameters were examined after grouping the data according to whether the temperature was constant or fluctuating (Figure 2.12). Group 1 (constant) comprised the hatching data from the positions where the minimum temperature differs from the mean temperature by <1°C and group 2 (fluctuating) comprised the data at positions where the difference between the minimum temperature and the mean temperature is >1°C. Figure 2.12 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 any of the parameters though there are differences in the variability. It also indicates that there was no difference between constant and diurnal regimes.

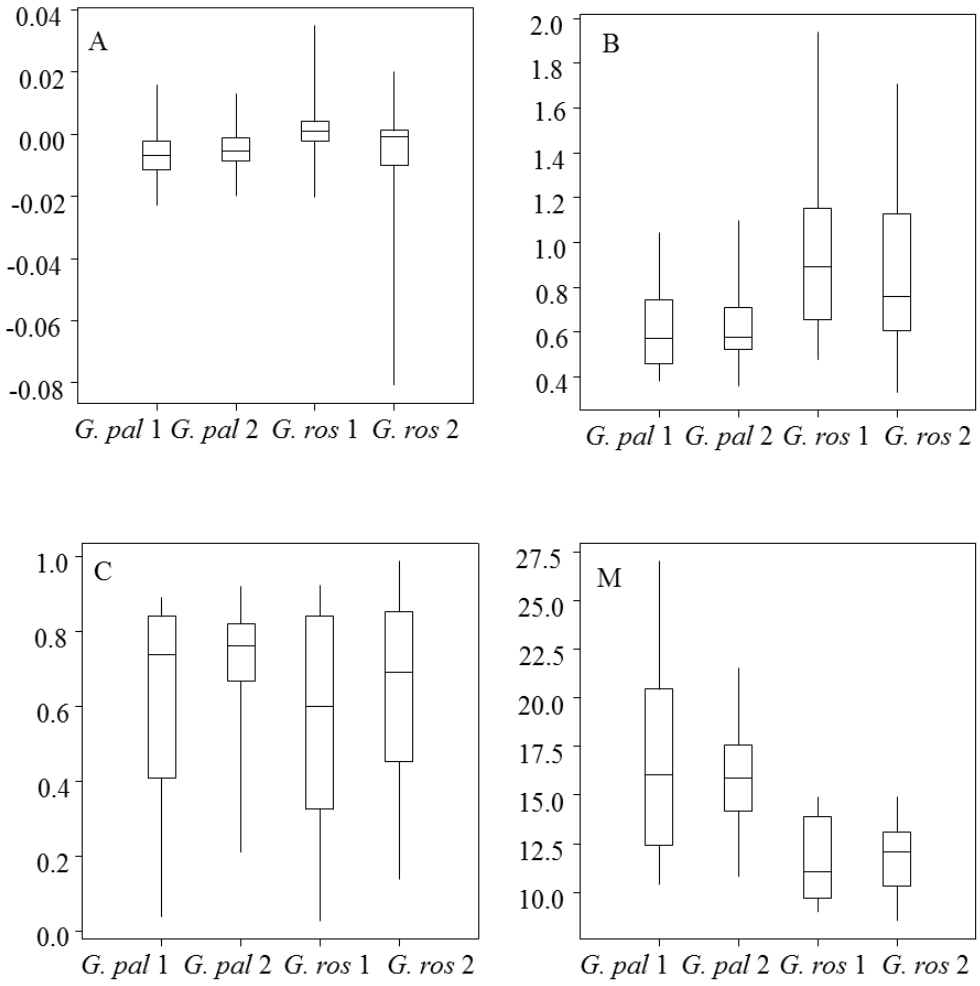


Figure 2.12 Boxplots of parameters A, B, C, M for logistic curves fitted to cumulative proportion of total hatch for *G. pallida* (*G. pal*) and *G. rostochiensis* (*G. ros*) grouped by (1) constant temperature regime, (2) fluctuating temperature regime.

The 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* at cooler mean temperatures. This is confirmed by the ANOVA test for M ($P<0.001$). Parameter B indicates that *G. rostochiensis* hatched at a faster rate than *G. pallida* at lower temperatures. 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 in the mean response at different temperatures (P<0.001) and for different species (P=0.005) as well as between temperature and species (P=0.026) (Figure 2.13).

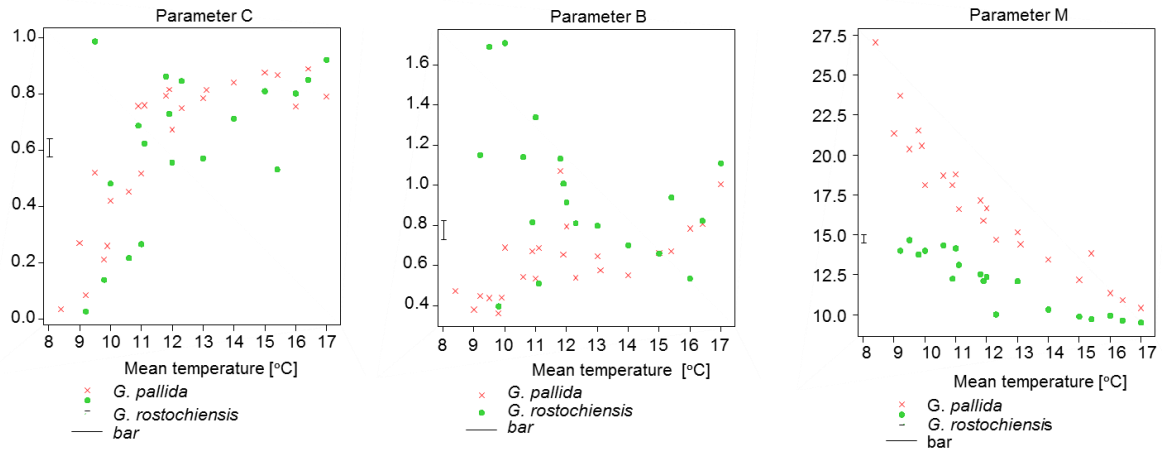


Figure 2.13 Comparison of the variation of the parameters C, B and M in the logistic model in terms of temperature in the diurnal temperature regime experiment for both PCN species (*Globodera pallida* (red) and *G. rostochiensis* (green)).

2.4.2. Female canister experiment

The differential effect of temperature on the development of PCN females is shown in Figure 2.14 and Figure 2.15. The first females of *G. pallida* on the roots of cv Desirée were observed 4 weeks after inoculation at the temperatures 20°, 22° and 18°C respectively. Female nematodes started appearing One week later, at 16° and 14°C. The latest first occurrence was recorded on the roots at 10°C, 9 weeks after inoculation and numbers of females continued rising until the end of the experiment at this temperature. The maximum number

of females developed between weeks 5–9. The highest number of females was recorded on cv Desirée at 14, 16 and 18°C.

There were significantly fewer numbers of females on cv Vales Everest compared to cv Desirée ($P<0.001$), however they also first appeared (Figure 2.16Figure 2.17) at 18°C, 20°C and 22°C. The maximum number of females was recorded between 5–9 weeks after inoculation. Moreover at 10°C with cv Vales Everest.

The numbers of *G. rostochiensis* females were relatively low compared to those observed with *G. pallida.* The first peak of females was observed in week 5 at 22°C on cv Desirée. The highest numbers of new females was recorded at 18°C and 20°C between week 7 and 8 (Figure 2.18). At the low temperatures of 10°C -12°C on cv Maris Piper, the number of females observed was close to zero (Figure 2.19).

Repeated Measurements ANOVA was applied with cultivar, temperature and time as factors to compare the impact of temperature on female development. For both species the comparison of the number of females on different cultivars revealed significant differences ($P<0.001$) in development at different temperatures. The differences in occurrence times due to varying temperatures were also significant ($P<0.001$).

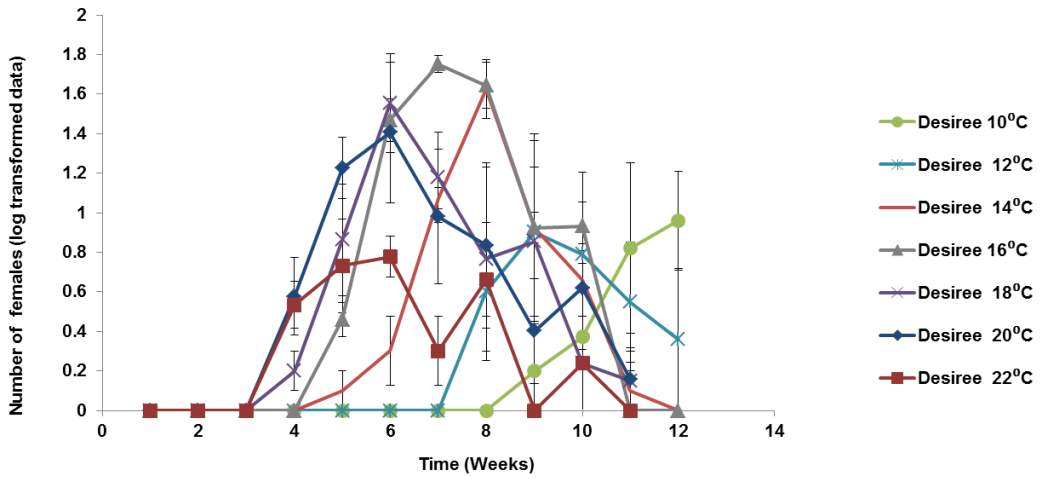


Figure 2.14 Appearance of females of *G. pallida* over 12 weeks incubation at 7 different temperatures (10–22°C) on cv Desirée in a canister test. The bars indicate the standard error of means for 3 replicates.

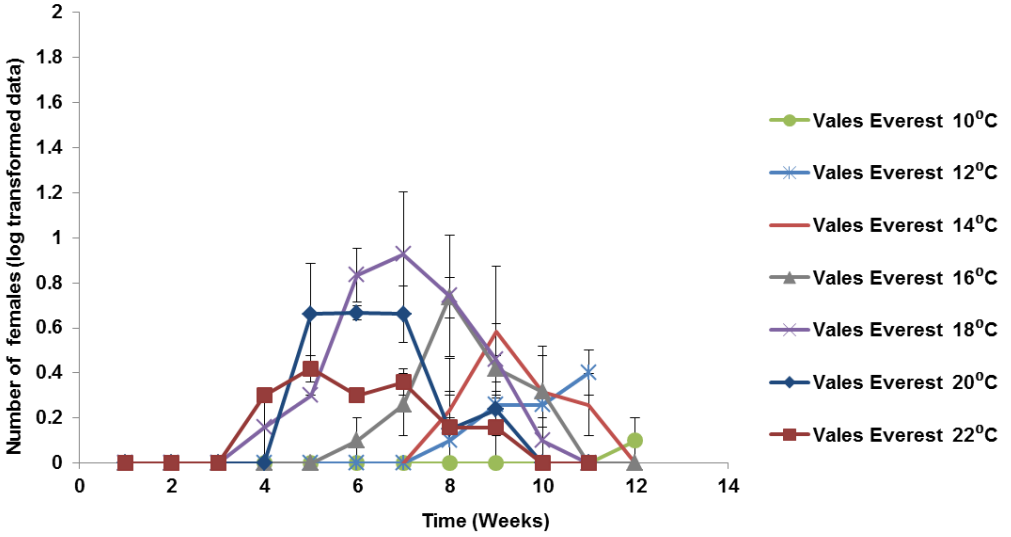


Figure 2.15 Appearance of females of *G. pallida* over 12 weeks incubation at 7 different temperatures (10–22°C) on cv Vales Everest in a canister test. The bars indicate the standard error of means for 3 replicates.

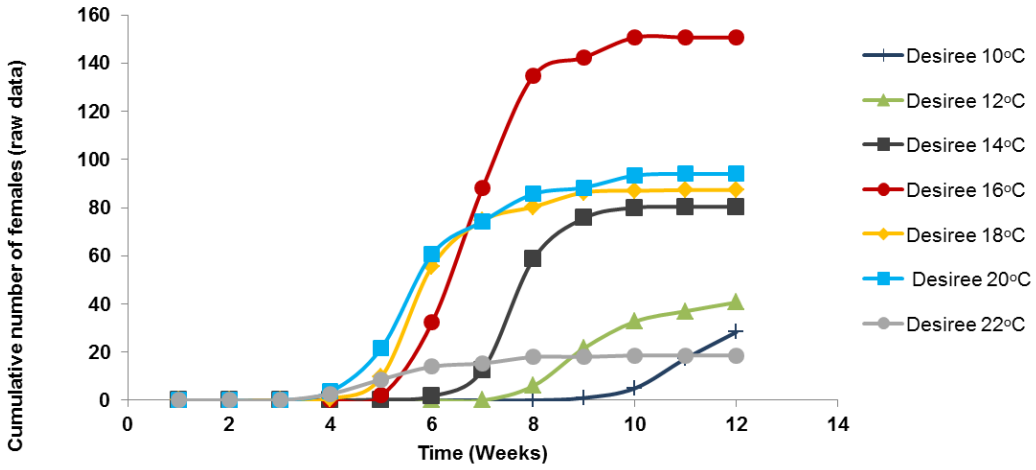


Figure 2.16 Cumulative number of females of *G. pallida* observed over 10 weeks at different temperatures (10–22°C) on the cv Desirée. Data are expressed as the accumulation of females that have developed at a particular temperature and are means of 3 replicates.

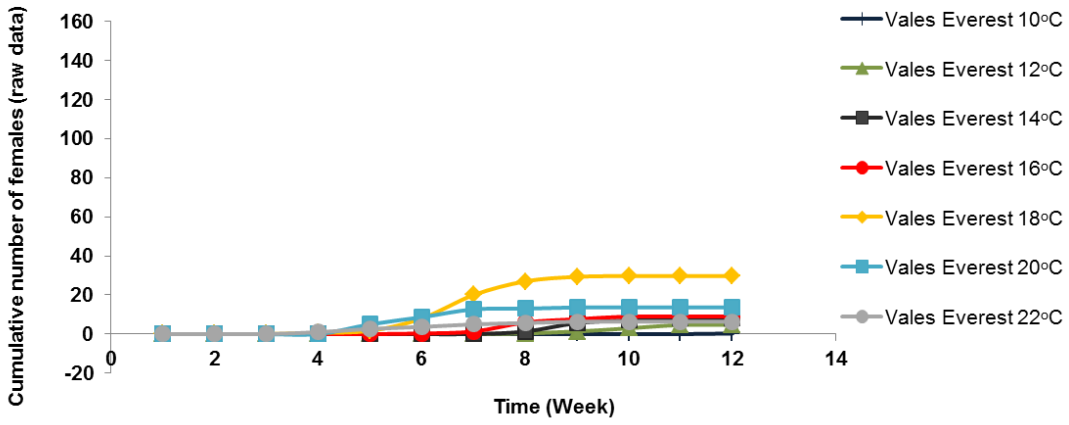


Figure 2.17 Cumulative number of females of *G. pallida* observed over 10 weeks at different temperatures (10–22°C) on the cv Vales Everest. Data are expressed as the accumulation of females that have developed at a particular temperature and are means of 3 replicates.

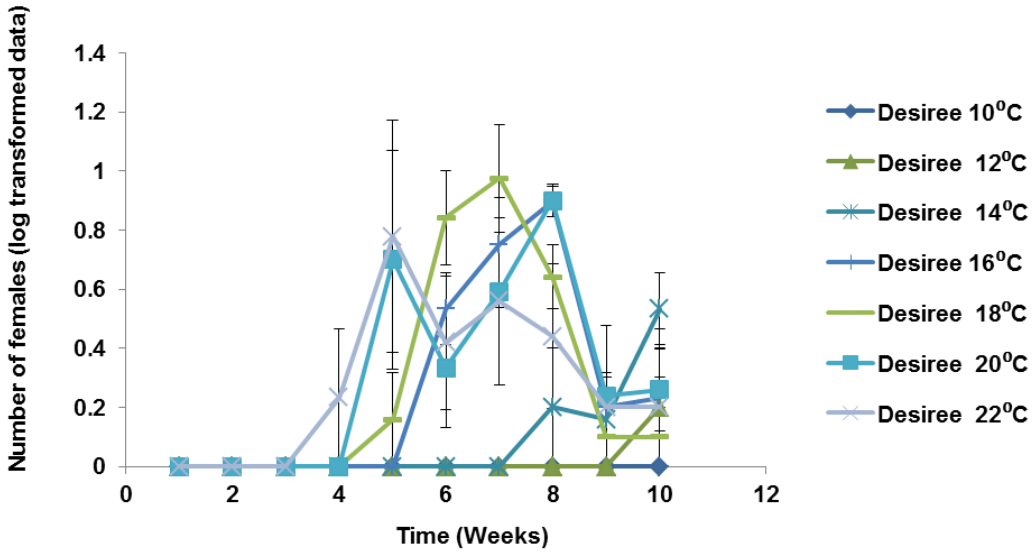


Figure 2.18 Appearance of the females of *G. rostochiensis* over 10 weeks of incubation at different temperatures (10–22°C) with cv Desirée in a canister test. The bars indicate the standard error of means for 3 replicates.

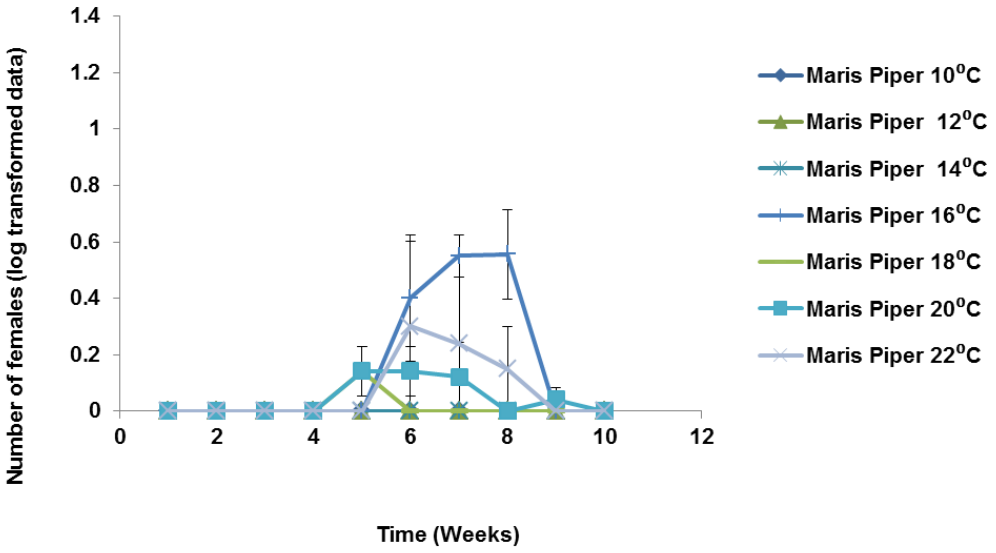


Figure 2.19 Appearance of the females of *G. rostochiensis* over 10 weeks of incubation at different temperatures (10–22°C) with cv Maris Piper in a canister test. The bars indicate the standard error of means for 3 replicates.

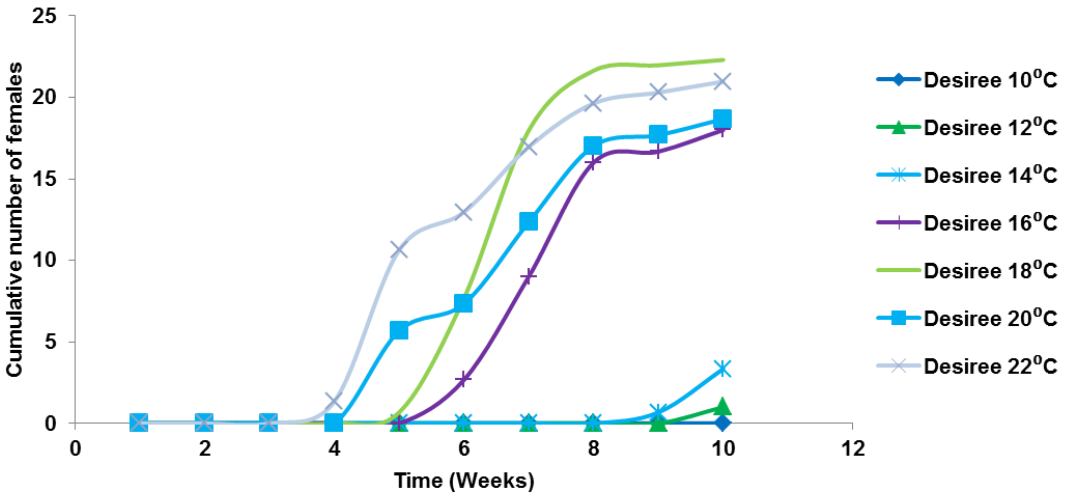


Figure 2.20 Cumulative number of females of *G. rostochiensis* observed over 10 weeks at different temperatures (10–22°C) on the cv Desirée. Data are expressed as the accumulation of females that have developed at a particular temperature and are means of 3 replicates.

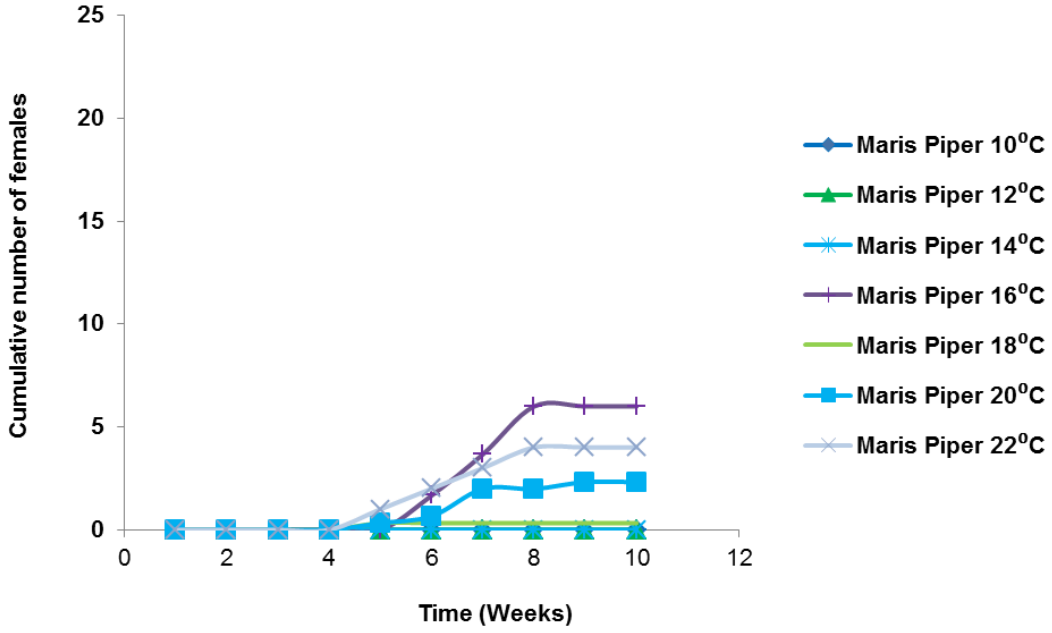


Figure 2.21 Cumulative number of females of *G. rostochiensis* observed over 10 weeks at different temperatures (10–22°C) on the cv Maris Piper. Data are expressed as the accumulation of females that have developed at a particular temperature and are means of 3 replicates.

2.4.3 Growth cabinet experiment

In vivo assays confirmed that there is an interaction between temperature and the life cycle of potato cyst nematodes. Temperature affected the duration of their life cycle and the numbers of individuals that were detected.

2.4.3.1 Occurrence of juveniles in the soil in different temperatures

For *G. rostochiensis* nematodes were recovered from the soil at week 2 and the first peak of hatched J2s was detected 3 weeks after inoculation. The highest peak was recorded at 18°C and then 11°C and 14°C respectively (Figure 2.22). No differences were found between numbers of hatched juveniles at 11°C and 14°C. Similarly, the number of nematodes did not differ at 14°C and 18°C. However, between 11°C and 18°C the numbers of hatched juveniles recovered from the soil differed. Also an increase in the number of juveniles recovered from the soil was recorded at week 9 in 18°C and at week 11 at 14°C.

The second growth cabinet experiment with *G. pallida* showed a similar relationship between temperature and PCN development. The highest numbers of juveniles recovered from the soil were recorded between weeks 2–4. The numbers of nematodes recovered from the soil was similar for both cultivars (Desirée and Morag) between weeks 1–5 at both temperatures. However, after 10 weeks the second hatch of juveniles was observed only with cv Desirée at both 17°C and in 14°C (Figure 2.23).

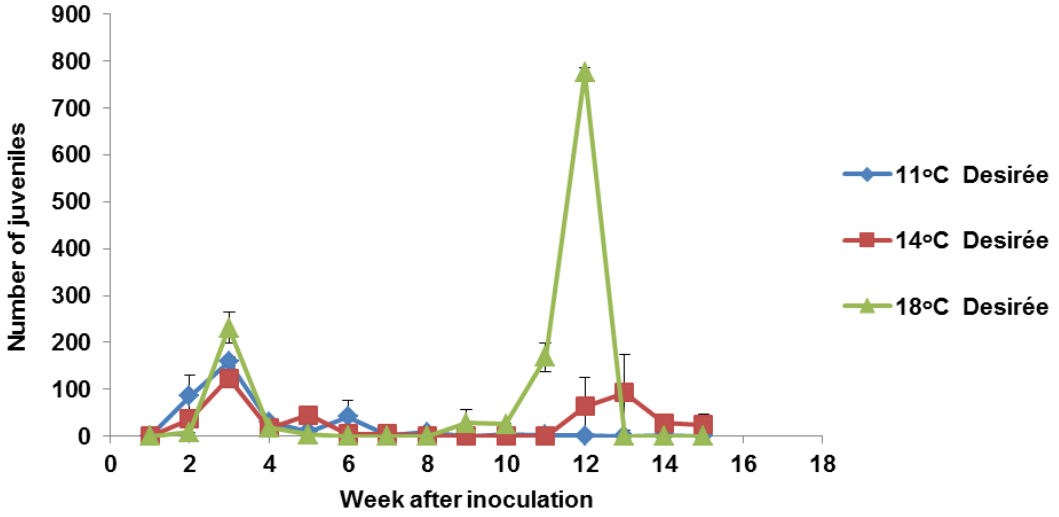


Figure 2.22 Number of juveniles *of G. rostochiensis* recovered from the soil over 12 weeks following inoculation in growth cabinets at average temperatures of 11, 14 and 18°C. The bars indicate the standard error of means.

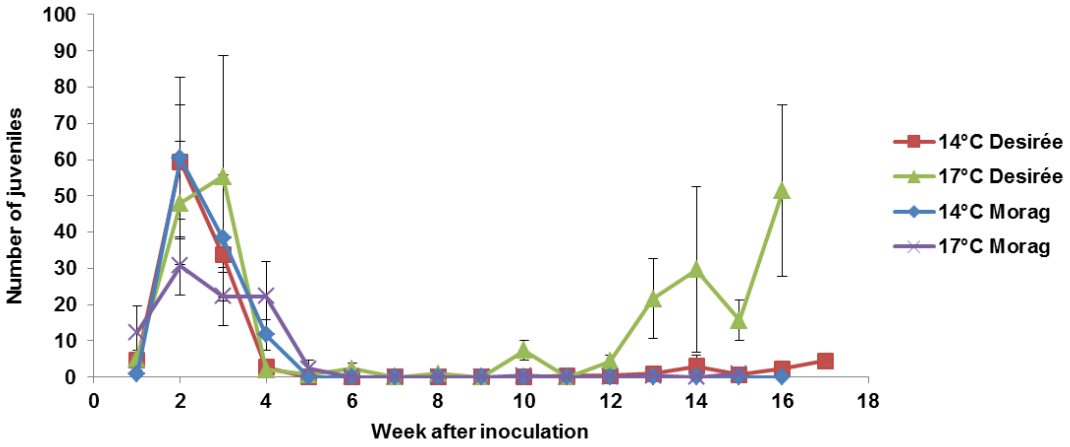


Figure 2.23 Number of juveniles of *G. pallida* recovered from the soil over 17 weeks following inoculation in growth cabinet experiment at 14°C and 17°C average temperatures with cvs Desirée and Morag. The bars indicate the standard error of means.

4.3.2 Occurrence of males in the soil in different temperatures

The first males of *G. rostochiensis* were recorded 5 weeks after inoculation at 18°C (Figure 2.24). The next males were observed at 11°C and 14°C in the 7th week. The highest number of males collected was noted at 18°C for *G. rostochiensis*, whereas for *G. pallida* the greatest peak was at 14°C on the cv Desirée (Figure 2.25). There was a delay in the occurrence of male at the lower temperatures. Similar results were obtained for both species of PCN. Strong influence of temperature was found in the response in the time delay. The cv Morag produced fewer males. A second smaller peak of *G. pallida* males appeared between the 14^{th} and 15^{th} weeks at 17°C on cv Desirée .

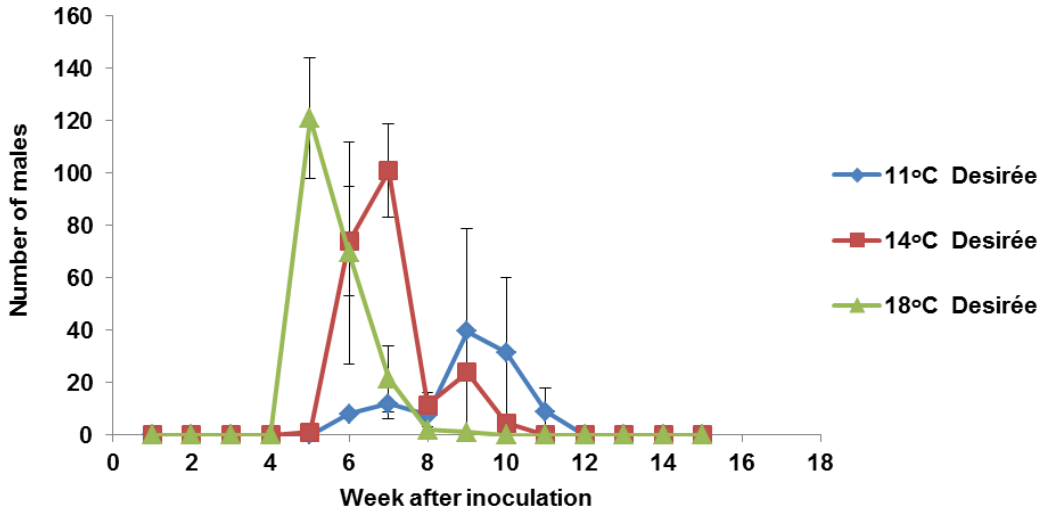


Figure 2.24 Number of males *of G. rostochiensis* recovered from the soil over 11 weeks following inoculation in the growth cabinet experiment at 11°C, 14°C and 18°C with cv Desirée. The bars indicate the standard error of means.

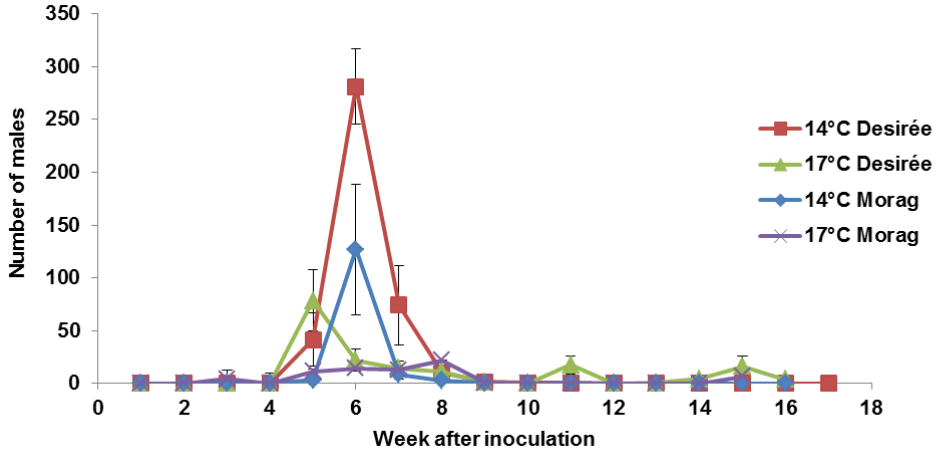


Figure 2.25 Number of males *of G. pallida* recovered from the soil over 16 weeks following inoculation in the growth cabinet experiment at 14°C and 17°C with cv Desirée and Morag. The bars indicate the standard error of means.

4.3.3 Occurrence of cysts in the soil in different temperatures

The first brown cysts were observed in the soil from the *G. pallida* growth cabinet experiment on the 9th week after inoculation in 14 and 17°C with cv Desirée (Figure 2.26). The highest number of cysts was observed 10 weeks after inoculation at 17°C and 15 weeks at 14°C. For the number of cysts there were significant differences in the mean responses at different temperatures ($P=0.005$), and for different cultivars ($P<0.001$), and there was also an interaction between temperature and cultivar ($P=0.007$).

The number of eggs per cyst from cv Desirée was significantly higher than from cv Morag (Figure 2.27) ($p<0.005$). However, there was no increase of egg content associated with the temperature ($P=0.273$).

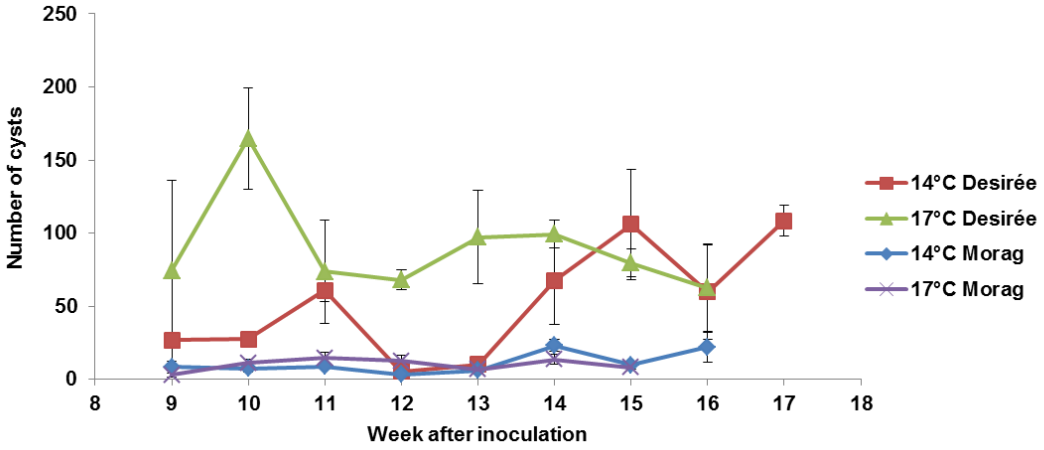


Figure 2.26 Number of cysts of *G. pallida* recovered from the soil over 17 weeks following inoculation in growth cabinet experiment at 14°C and 17°C with cv Desirée and Morag. The bars indicate the standard error of means.

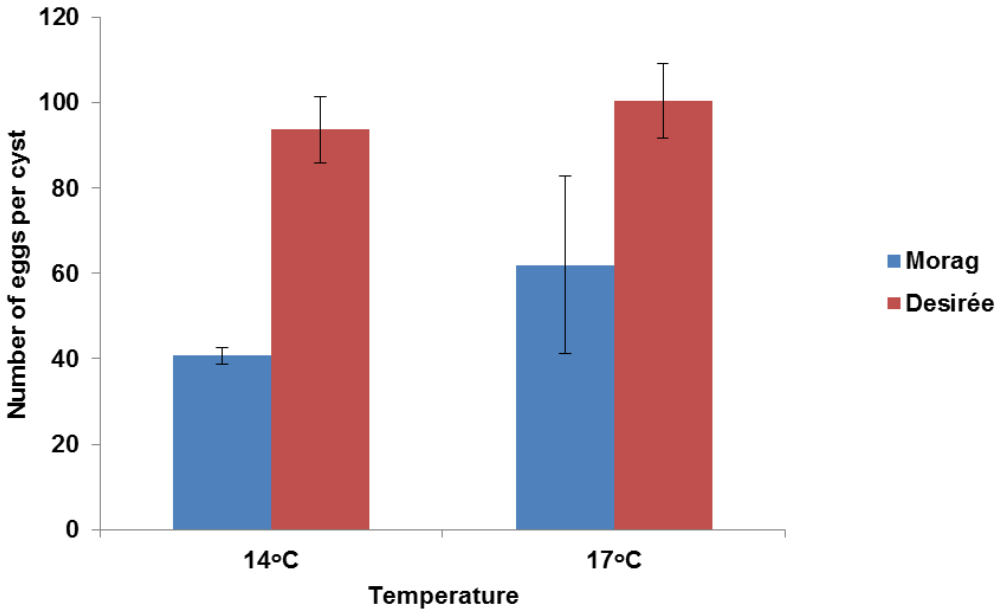


Figure 2.27 Average number of *G. pallida* eggs per cyst at 14°C and 17°C on cvs Desirée and Morag in growth cabinet experiment. The bars indicate the standard error of means.

4.3.4 Cyst size

The size and volume of newly formed cysts of *G. pallida* were established by taking pictures of the cysts collected from cvs Desirée and Morag in the growth cabinet experiment (Figure 2.28 and Figure 2.29).

An ANOVA was applied with cultivar and temperature as factors to compare the impact of temperature on cyst sizes. The comparison revealed significant differences between the cultivars ($P<0.001$) in cyst size and volume, however there were no significant differences between the temperatures ($P=0.246$) or interaction between temperature and cultivar ($P=0.156$).

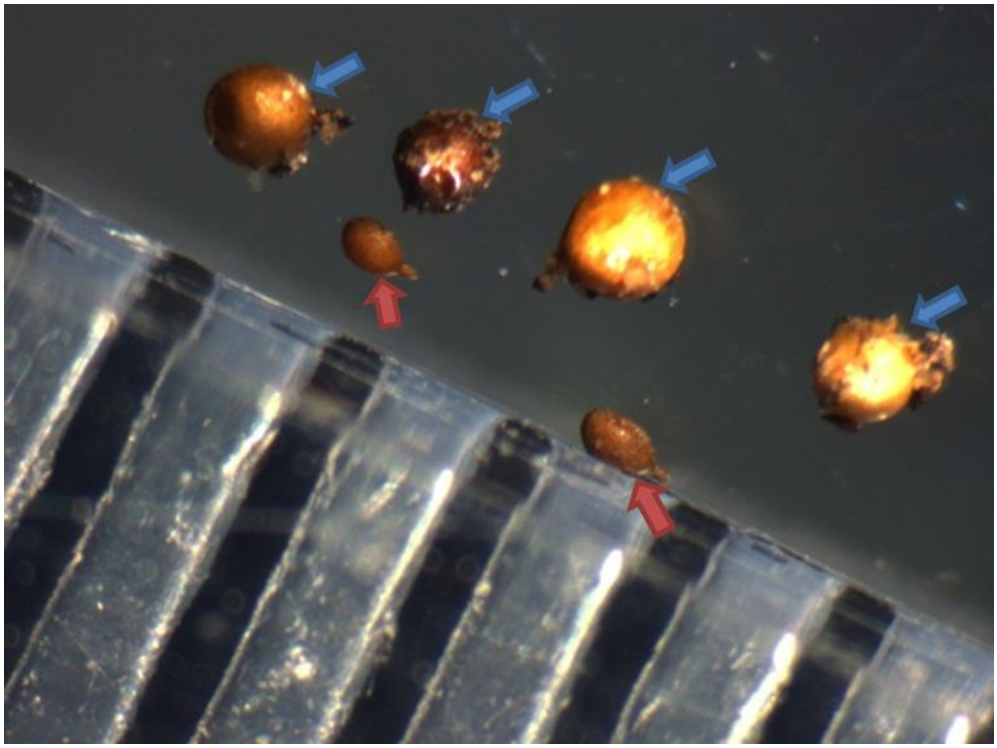


Figure 2.28 Cysts of *G. pallida* recovered from the soil 10 weeks after inoculation from the growth cabinet at 17°C. The red arrows show cysts recovered from cv Morag and the blue arrows cysts from Desirée.

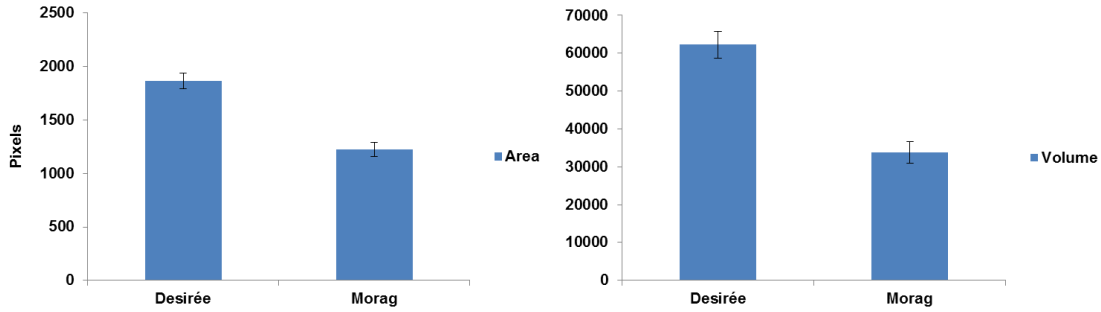


Figure 2.29 Average area (left) and volume (right) in pixels of 10 cysts of *G. pallida* from cvs Desirée and Morag. The bars indicate the standard error of means.

2.5. Discussion

Temperature regulates the metabolic rates of plant parasitic nematodes and their rates of development are perturbed when the temperature drops close to the basal threshold or increases above the optimum. Therefore it is an important factor affecting population dynamics of PCN that should be taken into account with regard to management strategies. The main goal of the experiments presented in this Chapter was to investigate the relationship between the temperature and the life cycle of potato cyst nematodes, and its influence on their development on the host plants with different resistance level at temperatures similar to field conditions in order to assess the risk of a second generation of PCN within one growing season.

The hatching experiments were designed to investigate the differences in the hatching reactions of *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 lowest observed temperature for hatching for both PCN species was 5°C. The maximum hatch in this temperature was about 5% which is similar to results that were recorded by Tiilikkala (1987) who observed the first second stage juveniles of *G. rostochiensis* in the soil at 4–5°C in Finland. In contrast, Mulder (1988) reported that hatching stops below 9°C, unlike, results from this

study show 35% hatching by *G. pallida* below 9°C. The data obtained also showed that little hatching of *G. pallida* occurred below 7°C and was on the level of spontaneous hatch in water.

Franco (1979) observed that *G. pallida* is better adapted to lower temperatures than *G. rostochiensis* and this is confirmed in the results presented here. The highest cumulative hatch for *G. rostochiensis* occurred at 21°C, which is comparable to Robinson *et al.* (1987) who noted a peak of newly hatched juveniles of *G. rostochiensis* at 20°C. His results also revealed that *G. rostochiensis* showed a significant increase in hatching with temperatures rising from 15–20°C. In contrast to *G. rostochiensis, G. pallida* had a broader temperature range over which similar amounts of hatching were observed. The highest amount of hatching for *G. pallida* occurred between 13–25°C. The raw data as well as curve fitting parameters suggest that *G. rostochiensis* generally hatches more quickly than *G. pallida*, however *G. pallida* showed a greater total hatch.

The second set of hatching experiments were conducted with a diurnal temperature cycle to investigate whether there were differences in the hatching responses of PCN in regimes simulating the fluctuations of the temperature that can occur in the field compared to hatching in constant temperatures. Hatching rates at constant temperatures were contrasted to hatching rates at similar average temperatures that were achieved with fluctuating temperatures. The results obtained showed that constant and fluctuating temperatures were not significantly different for both species of PCN, though the species again showed differences in their hatching responses to temperature. Although the hatching

tests in the diurnal temperature regime did not show a significant difference between the same constant and fluctuating temperatures on hatching, this could be because of the way the data was combined in the analysis due to the limited replication possible, and therefore subtle variations might not have been detectable.

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 worth speculating that these differences in the proportions of unhatched eggs could also affect population decline rates and the role of temperature in decline rates of PCN merits investigation.

To compare hatching *in vitro* and *in vivo* the growth cabinet experiments were carried out at different temperature regimes and under conditions that allowed to investigation of PCN hatching in the soil. Juveniles of *G. rostochiensis* that hatched in soil were collected with Baermann funnels and reached a maximum at all three temperatures at 21 days after inoculation, in contrast to the *in vitro* hatching where the highest hatching occurred at 11 days after incubation in PRD at 11–13°C and after 7–11 days between 15–19°C. The delay in hatching that was observed in the soil may be explained by the physical and chemical barrier of the soil increasing the time required for penetration of root exudates into the cysts and their detection by the encysted eggs (Spence *et al*., 2008; Dalzell *et al.,* 2011). Juveniles of *G. pallida* juveniles were observed 2–4 weeks after inoculation in the *in vivo* assay and showed the greatest hatch between 9–18 days at temperatures 14°C and 18°C. Hatching took longer with *G. pallida*

than *G. rostochiensis* in the growth cabinet experiment in agreement with the results obtained with the *in vitro* hatching experiments. Both *G. rostochiensis* and *G. pallida* showed the maximum hatch in the third week after inoculation. Similar to the hatching experiments, the pot test showed that higher soil temperatures promoted hatching of both PCN species.

Surprisingly 9 weeks after inoculation a second hatch of J2s of *G. rostochiensis* began at 18°C which confirms the potential for skipping the diapause stage and starting a second generation. Similar results were obtained by Jimenéz-Pérez (2009), who observed a second hatch of *G. rostochiensis* 56 days after planting in Venezuela. Stanton and Sartori (1990) reported two peaks of juveniles at 10–40 and 110–140 days in a pot experiment with conditions simulating temperatures at field sites infested with *G. rostochiensis*. Greco (1988) found that at Avezzano in Italy, *G. rostochiensis* completed a second generation within one growing season in the soil temperatures 18–22°C, with a late maturing variety. However a second generation was not completed at Catania in cooler conditions and with an early maturing variety. Like *G. rostochiensis, G. pallida* started producing a second hatch in 17°C and 14°C on the susceptible cv Desirée 10 weeks after inoculation. The numbers of juveniles from the second hatch were higher between the temperatures 14°C and 17°C on cv Desirée. The initiation of a second generation was not observed on the partially resistant clone Morag. Some of the juveniles from the second hatch were able to reach maturity and produced a second peak of males of *G. pallida* after 11 and 15 weeks on cv Desirée. From this, it could be hypothesised the second generation was nearly completed. However, it was not possible to investigate the further

development of the second generation because the potato plants were unhealthy causing the experiment to end prematurely. The results from the growth cabinet experiments support the conclusion that for both species of PCN, diapause is not obligatory in appropriate conditions.

As mentioned above this set of experiments examined also the effects of temperature on the development of the adult stages of PCN. The highest number of males of *G. rostochiensis* was observed at 18°C, and for *G. pallida* at 14°C in the growth cabinet experiments. The first males were observed 4 weeks after inoculation in both species of PCN and were recorded from 18°C, then 14°C and 11°C in weeks 5, 6 and 6 respectively with maxima in weeks 5, 7 and 10 respectively. The highest numbers were recorded at 14°C and 18°C. The first observed males were 5 weeks (35 days) after inoculation, which is consistent with Jiménez-Pérez (2009) who found a first peak of newly emerged males and females of *G. rostochiensis* in potato cv. Andinita 35 days at 18°C after inoculation in his experiment in Venezuela. In this study the first females of *G. rostochiensis* were observed in the canister experiment at 4 weeks at 22°C which suggest that the higher soil temperatures cause a faster life cycle for this species. However, *G. pallida* females were first observed at 16°C for Desirée after 4 weeks of inoculation. The appearance of the first females beginning at 4 weeks was earlier than the first appearance of males (both species 5 weeks) in the pot experiment; however, the recovery of males from the soil may have been less efficient than the direct observation of females on the roots and initially the number of females observed was very small.

The observations of females emerging on the roots in indicated that the most optimal temperatures for *G. pallida* development were 14–22°C and the optimal temperature for the overall numbers of females of *G. pallida* observed was 16°C for Desirée There were significant differences in the numbers of adult females recorded between 12°C and rest of the temperatures. Similar results were achieved with *G. rostochiensis*, though this species preferred the warmer temperatures. The numbers of females of *G. pallida* observed was greatly reduced with the partially resistant cultivar Vales Everest, though there was still an effect on the response at different temperatures. The appearance of the first females beginning at 4 weeks was earlier than the first appearance of males (both species 5 weeks) in the pot experiment; however, the recovery of males from the soil may have been less efficient than the direct observation of females on the roots and initially the number of females observed was very small. Although, these results differ from some published studies (Renco, 2007) who observed adult females filled with eggs 61 days after planting in temperatures 13–18°C, they are consistent with those of Jiménez-Pérez (2009) who recorded females 35 days after inoculation in 18°C.

Interestingly the numbers of eggs in the cysts from Desirée in both temperature regimes were not significantly different and the egg content of cysts from cv Morag was significantly lower. Bendezu *et al*. (1998) and Da Cunha *et al*. (2012) showed no significant reduction in the number of cysts or in egg content with more virulent populations of *G. pallida* from Portugal and Bolivia, however they also showed that less virulent populations can have significant differences in their multiplication with this source of resistance. Also, the initial hatching was

not affected by the cultivar used. The results from the growth cabinet experiments show that the partially resistant cv Morag did not affect the initial hatching. This is consistent with Turner (1990) who observed no significant difference in hatching on *S. vernei* hybrids. However, the present findings indicated reduction of the numbers of males and also an increase in the length of the life cycle. Moreover egg content within a cyst was also significantly higher in cv Desirée than in Morag suggesting the partial resistance from *S. vernei* had an influence on the number of eggs that were produced. Results obtained by Turner (1990) and Phillips and Trudgill (1998) on the same population as used in this study confirmed that partial resistance from *S. vernei* in Morag affected the reproduction of PCN.

The data presented also signifies that the hatching response is greater and the life cycle is faster at the optimal temperatures tested and thus increases in soil temperatures due to regional climatic differences or climate change are likely to favour PCN multiplication. However as demonstrated in the hatching tests and reported by Greco (1988) high soil temperatures might also adversely affect multiplication of PCN.

The two populations used in this study have been maintained in lab conditions over several generations and had been multiplied and stored under well-defined environments. According to Hominick (1982), environmental conditions influence *G. rostochiensis* females during development on potato roots, affecting the subsequent hatching of second stage juveniles. However, due to the limited replication in the growth cabinet experiments further statistical tests on numbers of juveniles and males estimating the probability value for these

was not possible, thus some interactions between temperature and juveniles or males were not detected.

Nevertheless the two species were found to differ in their responses to temperature as previously reported. These results are consistent with those from previous studies and it can be concluded that temperature affects the life cycle of potato cyst nematodes and an implication of this is the possibility that an increase of soil temperatures of 2°C would significantly speed up the life cycle of PCN and increase level of infestation in the field.

The differences in the responses of the two species to temperature has implications for interspecific competition between them when both occur as mixtures in the field, the host response to mixed infections and the composition of the final PCN populations. Differences in the response of PCN to different temperature regimes compared to other reports could be due to the adaptation of the nematode cultures to warmer conditions in the glasshouses or due interspecific differences of the original isolates, therefore need to be confirmed with populations from the field.

The survey done by Minnis *et al.* (2002) reported that mixtures of the 2 species were found in 25% of the fields he sampled in England and Wales. The presence of mixtures of the 2 species complicates the prediction of how temperature will impact on PCN population dynamics as interspecific competition between the species is likely to be density dependent and affected by temperature.

An implication from presented observations of the responses of the different life stages to temperature is that possible rises of soil temperatures would speed up

the life cycle of PCN and affect the amount and the speed of field population multiplication and increase level of infestation in the potato field.

3. **FIELD EXPERIMENTS**

3.1. Introduction

To manage populations of PCN in the field it is necessary to understand the population dynamics of these nematodes. Several models (Jones and Kempton, 1978; Phillips *et al*., 1991; Seinhorst 1967; Elliott *et al*., 2004) have been developed which illustrate the relationship between different factors (i.e. cultivar, soil type, nematicide treatment, tolerance, and initial Pi) and PCN population dynamics and yield loss, and these are being used to assist growers in their management decisions. The importance of different factors that affect PCN population dynamics has been investigated through many years of field trials and analyses of the resulting data. A better understanding of other factors that affect PCN population dynamics in different agroecological conditions could benefit the development of these integrated pest management systems as selection and timing tools of sustainable crop management. The control of PCN is likely to be much more effective when methods are combined in integrated control programmes. These include selective use of non-resistant cultivars that permit relatively little multiplication, the use of trap crops and biological control agents (Evans, 1993). It may be necessary to alternate resistant and non-host crops to prevent the selection of virulent nematodes on resistant cultivars from avirulent populations (Minnis *et al*., 2002; Turner and Fleming, 2002). With loss of nematicides due to changes in EU legislation and the increasing distribution of *G. pallida* for which there are few cultivars available with high levels of

resistance predicting tools such as population dynamic models are becoming more and more important.

The population dynamics of *G. pallida* and *G. rostochiensis* and the associated yield losses of the potato crop differ greatly between years and locations (Greco *et al*., 1982; Seinhorst, 1982). These differences may be the result of the initial population of PCN in the field (Trudgill *et al*., 2014), environmental factors such as temperature, soil type (Trudgill, 1986) or cultivar tolerance and resistance (Trudgill and Cotes, 1983; Elston *et al*., 1991; Trudgill, 1991). It has been reported (Seinhorst, 1965; Seinhorst, 1980; Greco and Di Vito, 2009; Trudgill *et al*., 2014) that in general the larger the population of *Globodera* spp. in the field at planting, the greater yield losses. Other authors (Ellenby and Smith, 1975; Franco, 1979; Mulder, 1988; Stanton and Sartori, 1990; Munir *et al.,* 2009; Blok *et al*., 2011) have noted that PCN populations are highly affected by the soil temperatures.

Multiplication of PCN is density dependent and the initial infestation (Pi) is negatively correlated with the rate of potato cyst nematode reproduction at high infestation levels (LaMondia and Brodie, 1986) due to juveniles of one or both species competing for feeding sites (Trudgill *et al*., 1992).

The two species of PCN are closely related and exploit the same ecosystem. In the previous chapter interspecific differences between *G. pallida* and *G. rostochiensis* in their rates of hatch and development in relation to temperature were described. Their interaction may be direct (interference competition) or indirect, for example competing for the same food source and resulting in a lack of available nutrients (den Nijs, 1992). Competition between nematodes might

be the result of lack of host availability, environmental factors (temperature) or the density of the competing populations (Eisenback and Griffin, 1987). The interaction between potato cyst nematodes is assumed to be antagonistic (den Nijs, 1992) as they share the same host plant.

Climatic conditions differ around the UK and there is a trend towards increasing temperatures and changes in rainfall associated with climate change (Parker *et al*., 1992; Jones *et al*., 2007) that is likely to impact on the multiplication and damage caused by PCN. The two sites chosen for field experiments in Scotland and England were selected so that they were widely geographically separated and thus likely to differ in their soil temperature profiles and to support the development of the Potato Council's PCN management model (Elliott *et al*., 2004).

3.2. Aims and Objectives

The first aim of this chapter was to examine the interaction between the two PCN species when they occur as a mixture by comparing their multiplication as individual species and as mixtures to assess whether interspecific competition affects PCN population dynamics when mixtures of the 2 species occur in the field.

The second aim was to determine the duration of the potato cyst nematodes life cycle in field conditions and to investigate the potential for more than one generation per year with different initial population densities, temperature regimes and agroecological conditions.

3.3. Materials and Methods

3.3.1. Nematodes

Cysts from *G. rostochiensis* A (pathotype Ro1) and *G. pallida* E/Lindley (pathotype Pa2/3) populations from the James Hutton Institute PCN collection were stored at 4°C for at least one year prior to use. Cysts were randomly selected following sieving (250 µm) to exclude small and damaged cysts. To produce the egg inoculum, cysts were homogenized with a Citenco Homogenizer (Jencons Scientific Ltd.) in 250 ml of sterile distilled water to achieve an inoculum density of 80 eggs/g. For the estimation of the egg concentration, three counts were made.

Experimental plots for the field experiment were naturally infested by PCN. The species and initial Pi was determined by qPCR (see below).

3.3.2. Location of field trials

Field trials took place in 2 locations: Luffness Mains near Aberlady, East Lothian in Scotland (56.0170° N, 2.8375° W) , and near Harper Adams University College near Newport, Shropshire, England (52.7797° N, 2.4275° W). in 2011 and 2012. Due to crop rotation, field experiments in 2012 were carried out at neighbouring experimental plots with different levels of initial population of PCN.

3.3.3. Plant material

The *Solanum tuberosum* cultivars used in pot experiments to examine interspecific competition were the susceptible cv Desirée and cvs Vales Everest

(*S. tuberosum* spp. *andigena* CPC 2802 partially resistant to *G. pallida*) and Maris Piper fully resistant to *G. rostochiensis* (*H1*).

For the field experiments four cultivars were planted at each site, cvs Desirée, Maris Piper, Cara and Estima and Edzell Blue was used as guard plants to reduce edge effects. The cultivars selected represent cultivars that are commonly grown in the UK and are early maturing (Estima), main crop (Desirée and Maris Piper (H1)) and late cropping (Cara(H1)). In the second year cv partially resistant to *G. pallida,* Vales Everest (*S. tuberosum* spp. *andigena* CPC 2802) was substituted for Estima.

3.3.4. Interspecific competition experiment

To examine competition between *G. pallida* and *G. rostochiensis,* tuber pieces of cvs Desirée, Vales Everest or Maris Piper were planted in pots containing 260 g sand:loam (50:50) which had previously been autoclaved and mixed. A single potato sprout on a spherical piece of tuber cut from a seed tuber was planted in each pot. Plants were grown in the greenhouse until they were ~10 cm above ground. The pots were placed in pallets on top of a layer of autoclaved sand in a randomized design in the glasshouse. One week after planting potato plants were inoculated with PCN in the combinations shown in Table 3-1. After 10 weeks, watering of the plants was stopped, the soil was allowed to dry and then packaged in paper bags, labelled and transferred to SASA for cyst extraction using the automated soil washing carousel (Meku, Germany). The paper filters on which the floats from washed soil were recovered were dried and the cysts were further purified by acetone flotation

(Brodie *et al*., 1976) at The James Hutton Institute. The total number of cysts was counted using a stereomicrosope (Olympus S7-ST). Next the cysts were transferred into a 2 ml Eppendorf tube and DNA extraction and qPCR was performed to determine the species composition in the mixtures at the end of the experiment as described in section 3.3.10.

3.3.5. Experimental design of field trials

The field experiments were designed to have six harvests with the final harvest also used for determining the final yield for each of the four cultivars and treatments (with or without nematicide) at each site. Within each harvest area the five replicates of the four cultivars were randomised (Table 3-2). Each replicate consisted of 3 tubers planted 25 cm apart. Between individual harvest areas, the final yield areas and also at the end of each row, two guard plants (Edzell Blue) were planted to minimize edge effect.

The nematicide treatments used for the field experiments in 2011 were: at Luffness 55 kg/ha of Vydate and in Harper Adams fosthiazate at 30 kg/ha. In 2012 in both sites Vydate (55 kg/ha) was used. The farmer was responsible for all the routine agricultural operations, including nematicide application, fertilisation and application of other crop protection products at each site.

Table 3-1: Different concentrations of the initial populations (Pi) of PCN in eggs/g soil used as an inoculum for the pot competition experiment.

Initial population	Proportion of *G. rostochiensis* (A)	Proportion of *G. pallida* (E)
A	25 (20 eggs/g)	0
A	50 (40 eggs/g)	0
A	75 (60 eggs/g)	0
A	100 (80 eggs/g)	0
E	0	25 (20 eggs/g)
E	0	50 (40 eggs/g)
E	0	75 (60 eggs/g)
E	0	100 (80 eggs/g)
A + E	75 (60 eggs/g)	25 (20 eggs/g)
A + E	50 (40 eggs/g)	50 (40 eggs/g)
A + E	25 (20 eggs/g)	75 (60 eggs/g)

Table 3-2 An example of the randomization plan for the four cultivars (Desirée, Estima, Maris Piper and Cara) in one of the harvest areas (H1) for the field trials in 2011 and 2012 (G is guard plant and H is harvest).

G G
G G

H1-1 (3 plants of Desirée)	H1-11 ((3 plants of Estima)
H1-2 (3 plants of Maris Piper)	H1-12 (3 plants of Cara)
H1-3 (3 plants of Estima)	H1-13 (3 plants of Desirée)
H1-4 (3 plants of Desirée)	H1-14 (3 plants of Estima)
H1-5 (3 plants of Cara)	H1-15 (3 plants of Cara)
H1-6 (3 plants of Maris Piper)	H1-16 (3 plants of Maris Piper)
H1-7 (3 plants of Desirée)	H1-17 (3 plants of Desirée)
H1-8 (3 plants of Maris Piper)	H1-18 (3 plants of Estima)
H1-9 (3 plants of Cara)	H1-19 (3 plants of Cara)
H1-10 (3 plants of Estima)	H1-20 (3 plants of Maris Piper)

G G
G G

3.3.6. Soil sampling and harvesting

To estimate the initial PCN populations, 5 preplant composite soil sample cores (one preplant soil sample per four experimental plots) were taken in each harvesting area and final yield area. At both sites soil samples were collected at approximately monthly intervals: in Harper Adams from April to September in 2011 and 2012 and in Luffness from May to October in 2011 and 2012 (Table 3-3). During harvesting, soil around the roots from the 3 plants/replicate was combined in a bucket, mixed and then ~500gm was placed in a plastic bag. Soil samples were transferred to a tray within 24 h and dried in the greenhouse, then 400 g of dry soil sample was packaged in a paper bag for the cyst extraction at SASA using the automated soil washing carousel (Meku, Germany). The filters on which the floats were recovered were dried and the cysts were further purified by acetone flotation (Brodie *et al*., 1976) at The James Hutton Institute. The resulting floats were allowed to dry and then transferred into a 2 ml Eppendorf tube.

Table 3-3 Dates for each harvest in 2011 and 2012 at Luffness and Harper Adams.

Harvest	2011		2012	
	Luffness	Harper Adams	Luffness	Harper Adams
Harvest 0 (preplant sampling and planting)	03/05	03/04	03/05	01/04
Harvest 1	01/06 (4 weeks)	02/05 (4 weeks)	31/05 (4 weeks)	10/05 (5 weeks)
Harvest 2	28/06 (8 weeks)	30/05 (8 weeks)	04/07 (8 weeks)	07/06 (9 weeks)
Harvest 3	26/07 (12 weeks)	27/06 (12 weeks)	01/08 (12 weeks)	5/07 (13 weeks)
Harvest 4	24/08 (16 weeks)	22/07 (16 weeks)	05/09 (16 weeks)	02/08 (17 weeks)
Harvest 5 (yield)	30/09 (20 weeks)	24/08 (20 weeks)	16/10 (20 weeks)	06/09 (22 weeks)
Harvest 6 (yield)	30/09 (20 weeks)	01/09 (22 weeks)	16/10 (20 weeks)	05/10 (26 weeks)

3.3.7. Examination of roots for presence of PCN

In 2011 the root systems of cvs Desirée and Cara were collected from the field trials in Harper Adams and Luffness for monitoring nematode development at the same time that the soil samples for each replicate of 3 plants were taken. The root samples were stored in FAA (Formalin-Acetic-Alcohol) solution (Hooper, 1970) until they were stained with acid fuchsin.

FAA (Formalin-Acetic-Alcohol) (100 ml) contained:

Ethyl alcohol ---------- 50 ml

Glacial acetic acid ----------5 ml

Formaldehyde (37–40%) ----------10 ml

Distilled H_2O ---------- 35 ml

Roots were stained with the acid fuchsin by soaking for 2 minutes in diluted sodium hypochlorite solution (1%), then they were washed for 5 min with three changes of tap water. Washed roots were next transferred to a beaker of boiling 1X acid fuchsin stain (10x stock: 0.35% acid fuchsin made up in 25% glacial acetic acid) and left in stain for 2 minutes. Stained roots were rinsed briefly in tap water and placed in a dish containing acidified glycerol (1 drop of glacial acetic acid per 100 ml glycerol). Nematodes were examined under microscope (Olympus S7-ST), counted and the life cycle stage recorded. Pictures were obtained using a Leica M165C microscope with a Micropublisher camera controlled by QCapture Pro software.

3.3.8. DNA extraction from cysts

The floats obtained following acetone purification were pulverised in a mixer mill MM300 (Retsch) in 2 ml Eppendorf tubes with 2 metal beads/tube for 1.5 min at 30 Hz frequency. The resulting powder was mixed with 0.5 ml of GeneScan Lysis Buffer (Neogen Europe Ltd.) and ground again for 30s. Samples were centrifuged for 15 seconds at 15600 x g and then 5 µl of 20 mg/ml Proteinase K in 40% (v/v) glycerol (Sigma) was added and incubated for 1h at 65°C. After incubation, 500 µl chloroform/isoamyl alcohol (24:1) (Sigma) was added and mixed by inverting the tube several times. Samples were then centrifuged for 10 min at 15600 g in an Eppendorf centrifuge and the upper aqueous phase (450 µl) was transferred into a new tube and 360 µl of ice-cold iso-propan-2-ol (BDH) was added and mixed thoroughly. Samples were then incubated for 30 min at -20°C, centrifuged for 10 min at 15,600 x g or 25 min at 3300 x g and the pellet

retained. The pellet was washed twice with 500 µl 75% ethanol by centrifugation for 5 min at 15600 x g. Finally the pellet was dissolved in 100 µl sterile dH_2O.

The resulting DNA was further purified on PVPP columns as follows. A 600 µl suspension of 10% PVPP (Sigma) was made up with sterile distilled water. The suspension was transferred to an empty spin column (NBS Biologicals) in a 2 ml microcentrifuge tube. Tubes were then centrifuged at 11,000 x g for 1 minute. The catch tube was then emptied and the centrifugation repeated (the spin column was turned 180° within the centrifuge before the second spin). The resuspended DNA was transferred to the spin column and centrifuged at 11,000 x g for 1 min. Purified eluate was transferred to a new sterile 1.5 ml tube (Eppendorf) and stored in -20°C.

3.3.9. qPCR validation

The oligonucleotide design and optimization of assay was published by Reid *et al*. (2010). Two sets of primers and probes were used that were designed for the rDNA ITS1 region of *G. pallida* and *G. rostochiensis* (Table 3-4).

Table 3-4 Primers and probes sequences

Primer 1 (Forward)	CGTTTGTTGTTGACGGACAYA
Primer 2 (Reverse)	GGCGCTGTCCRTACATTGTTG
G. pallida MGB probe	6FAM-CCGCTATGTTTGGGC
G. rostochiensis MGB probe	6FAM-CCGCTGTGTATKGGC

Samples with known amounts of cysts of *G. pallida* (Lindley E2010) and *G. rostochiensis* (Ro1 A) were processed as described in section 3.3.8.

Real time qPCR reactions were set up using a Tecan Genesis Workstation 150 (Tecan Inc.) in 96 well plates (Applied Biosystems) at SASA. 30 µl reactions contained: 15.0 µl Environmental BLUE Ready Mix (Eurogentec Ltd), 1.25 µl each of the forward and reverse primers and probe for either species of PCN (at 5 pmol/µl), 1.25 µl of *G. pallida* specific probe at 5 pmol/µl, 1.25 µl of *G. rostochiensis* specific probe at 5 pmol/µl, 6.25 µl distilled water (Sigma) and 5 µl DNA from samples extracted as described above and diluted 1:10 with H_2O, or H_2O for the negative control. The reactions were then aliquoted in triplicate into 384 well plates (Applied Biosystems) using a Tecan Genesis robot. Standards (in triplicate) consisted of DNA that had been extracted from single cysts of both species and then diluted to 10000 pg, 1000 pg, 100 pg, 10 pg and 1 pg respectively. Amplification was performed in an ABI 7900HT (Applied Biosystems) real time machine run in the fast 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 (Reid, 2010). Linear regression of the qty values versus cyst number and calculation of the corresponding R^2 value were performed using Microsoft Excel software version 2010. The data obtained were logarithmically transformed to give normality.

To obtain the average number of eggs per cyst, 10 replicates of 3 randomly selected cysts were homogenised in a glass homogeniser (GPE Scientific, 20104) and the total number of eggs was counted with a stereomicroscope (Olympus S7-ST). The average egg content per cyst of *G. pallida* was 205.4 ± 20.3 and 233.9 ± 22.9 for *G. rostochiensis* (Table 3-5). The qty value obtained from the qPCR assay was converted to an egg number using the following

formula: number of cysts from samples used for validation were multiplied by the previously obtained average number of eggs/cyst, and the resulting number of eggs was then divided by the obtained qty value giving the result of an average of 246.44 eggs for *G. pallida* and 10.40 eggs for *G. rostochiensis* per 1 ng/µl.

3.3.10. Population quantification of samples from competition experiment

DNA from the competition experiment samples was extracted, diluted 1:1000 and used to determine the number of eggs/g soil from the qPCR quantity value. In order to establish the number of eggs per gram of soil, the number of eggs obtained from qPCR was divided by 260 grams (dry weight of the soil sample from the pot).

3.3.11. Population quantification of samples from field experiment

DNA from soil samples was extracted, diluted 1:10 and used to determine the number of eggs/g soil from the qPCR quantity value. In order to establish the number of eggs per gram of soil, the number of eggs obtained from qPCR was divided by 400 grams (dry weight of the soil sample from the field).

3.3.13. Soil temperatures

Soil temperatures were recorded during the field trials at a 20 cm depth in potato ridges with DS1920-F5 Temperature ibuttons (HomeChip, Milton

Keynes, UK). However in 2012 field experiment thermochrones set up at the Luffness site were damaged during the experiment and no data was collected. Additionally temperatures were recorded during the potato growing season at 14 sites around the UK between 2010 and 2013 including the experimental sites. Details of the locations together with planting and harvesting dates are included in Appendix 2.

3.3.12. Yield

Tubers from the last harvest at Luffness and Harper Adams in 2011 and 2012 were collected from each replicate and used to establish the final yield. Tubers from the three plants in each replicate were combined and sorted through riddles into size classes (>85 mm, 85–65 mm, 65–45 mm, <45 mm) and fresh weights were determined for each class. The number of tubers in each category was recorded and weighed and the total yield determined.

3.3.14. Statistical analysis

The results were transformed as necessary to normalise the data and analysed using GenStat Version 14.1 and Microsoft Excel Version 14.0.4760.1000. The data were analysed using an analysis of variance or an unbalanced design for analyses of variance.

3.4. Results

3.4.1. Histology of roots

The number of larvae recorded in the roots was transformed to achieve the number of nematodes per 100 g of roots (Figure 3.1). With the first harvest (week 4), nematodes were observed in the roots of both cvs Desirée and Cara from Harper Adams. They were identified as J2s. The highest number was recorded in cv Cara from the non-treated plots. Similarly, in the roots of cv Cara from harvest 1 from Luffness, the nematodes observed were J2. At harvest 2, J3 and J4 stage juveniles were observed in roots of cv Desirée from both treated and non-nematicide treated plots in Luffness while J3 were observed in roots of both cvs Desirée and Cara from Harper Adams. At harvest 3, J2 and J3 stages were observed in roots from cv Cara at Harper Adams in non-nematicide treated plots, while no nematodes were found in roots from the treated plots. At Luffness J2 were observed in both no nematicide and nematicide treated plots with cv Desirée planted. At harvest 4 at Luffness no juveniles were detected in contrast to Harper Adams, where J2s were observed in roots of cvs Cara and Desirée in both treatments. At Luffness it was not possible to inspect the roots as in the last harvest the roots were unhealthy and necrotic for both treatments and cultivars.

In Luffness the first occurrence of females in roots from cvs Cara and Desirée was recorded 8 weeks after planting (Figure 3.1 and Figure 3.2). Surprisingly the highest numbers were found with the nematicide treatment. Similarly with Desirée, the highest number of females was found with roots from the

nematicide treatment at 8 weeks. After 12 weeks females were still developing in roots of cv Desirée with nematicide treated plots. In week 16 there was no female development detectable. No roots were available after 16 weeks to observe because they had rotted. In the roots from the Harper Adams, females became visible 8 and 12 weeks after the potatoes were planted with higher numbers observed without nematicide treatment. The second appearance of females was recorded 20 and 22 weeks after planting on cvs Cara and Desirée respectively.

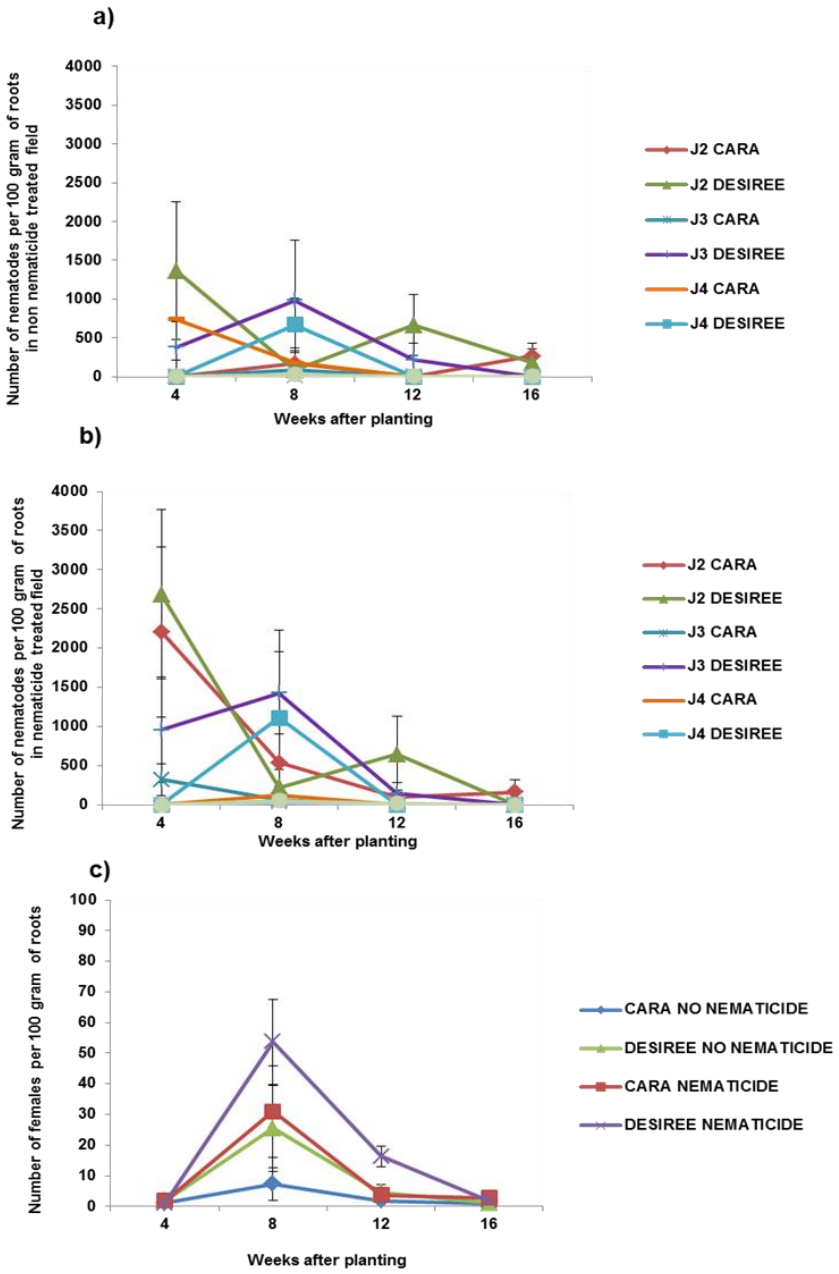


Figure 3.1 The numbers of J2, J3, J4, and females observed inside acid fuchsin stained roots of cultivars Cara and Desirée from 2011 field trials at Luffness a) non nematicide treated plots, b) nematicide treated plots c) females from both treatments Root samples were examined at Harvests 1, 2, 3, and 4 (weeks 4, 8, 12, 16,) The bars indicate the standard error of the mean.

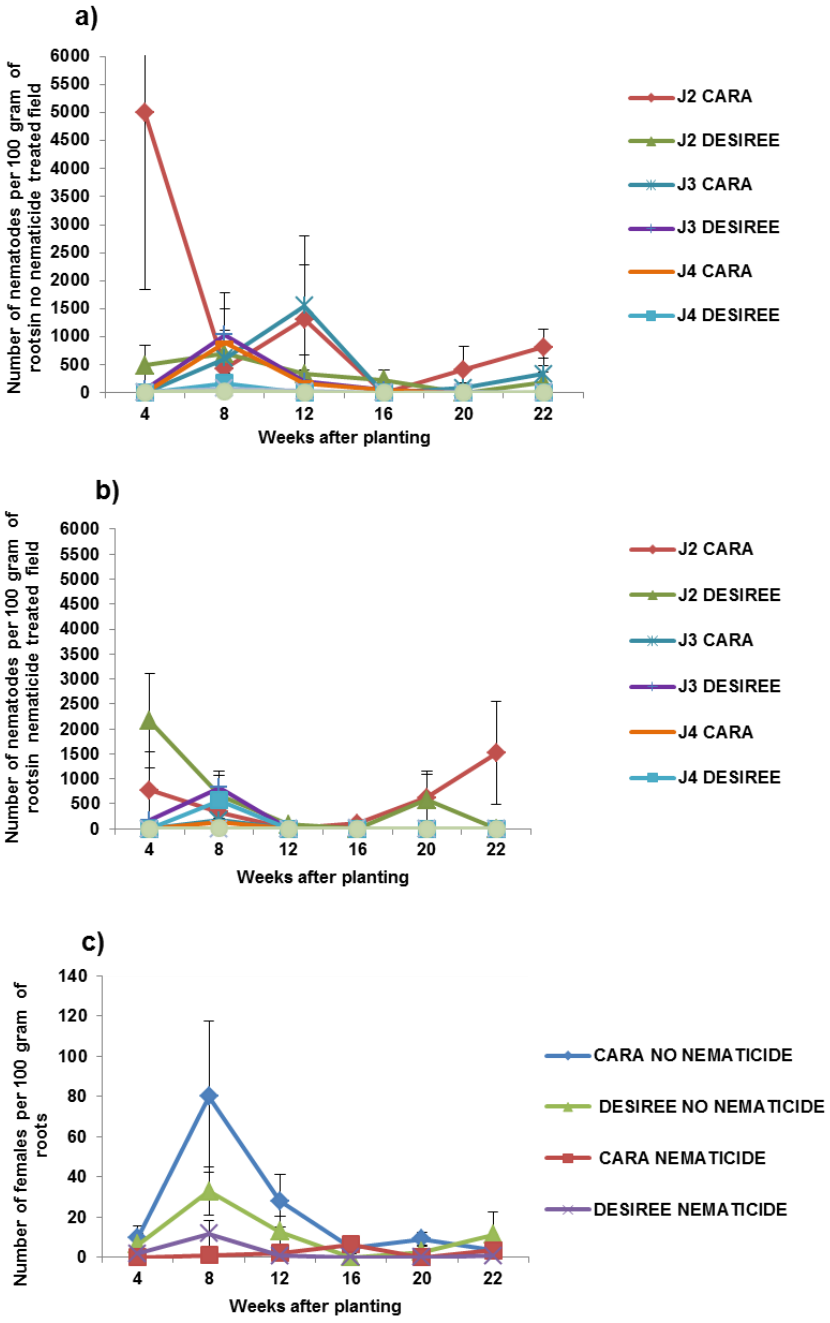


Figure 3.2 The numbers of J2, J3, J4, and females observed inside acid fuchsin stained roots of cultivars Cara and Desirée from 2011 field trials at Harper Adams. a) non nematicide treated plots, b) nematicide treated plots c) females from both treatments. Root samples were examined at Harvests 1, 2, 3, 4, 5 and 6 (weeks 4, 8, 12, 16, 20 and 22) The bars indicate the standard error of the mean.

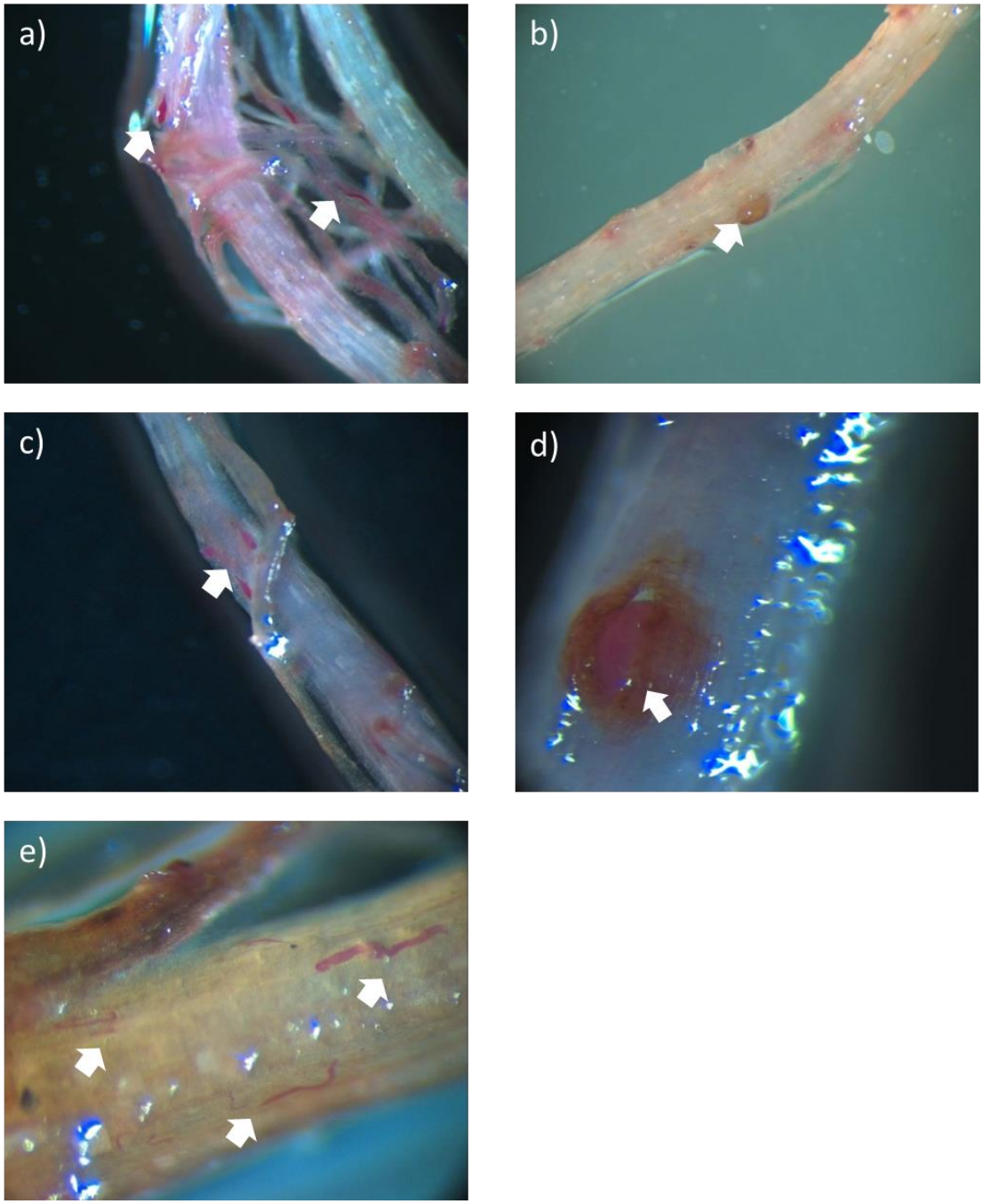


Figure 3.3 **Acid fuchsin stained potato cyst nematodes potato roots from the field experiments performed in 2011. White arrows show stained nematodes; a) J2 and J4 (found in root of cv Desirée 8 weeks after planting in Luffness in nematicide treated field, b) female found in root of cv Desirée 12 weeks after planting in Luffness in nematicide treated field, c) J4 found in root of cv Cara 8 weeks after planting in Harper Adams in non-nematicide treated field, d) female found in roots of cv Cara 8 weeks after planting in Harper Adams in non-nematicide treated field, e) J2 and J3 found in root of cv Cara 22 weeks after planting in Harper Adams in nematicide treated field.**

3.4.2. qPCR Validation results

The relationship between cyst DNA content obtained from qPCR and the number of eggs was established by carrying out qPCR using samples with known numbers of cysts and determining an average egg content/cyst. The DNA yield from *G. rostochiensis* and *G. pallida* from cysts was positively correlated with the number of cysts that were counted (Figure 3.4) (Log number of cysts of *G. pallida*= 0.6323x + 2.3574 x DNA yield; R^2 = 0.7717 and Log of number of cysts of *G. rostochiensis* = 0.5691x + 0.4324 x DNA yield; R^2 = 0.8632).It was determined that 1 ng/µl of *G. pallida* corresponds to 246.44 eggs and of *G. rostochiensis* to 10.41 eggs. The relationship between the estimated number of eggs from manual counting was positively correlated with the number of eggs estimated from qPCR (Figure 3.5).

Table 3-5 The number of eggs for 10 samples of 3 cysts from *Globodera pallida* and *G. rostochiensis* populations.

Sample	*G. rostochiensis*	*G. pallida* Egg content
3 cysts	694	856
3 cysts	726	962
3 cysts	854	512
3 cysts	529	685
3 cysts	795	653
3 cysts	380	733
3 cysts	1123	470
3 cysts	824	444
3 cysts	378	448
3 cysts	713	400
Mean of 3 cysts	701.6	616.3
Standard error of the mean	71.7	60.9
Mean of the number of eggs per single cyst	233.8	205.4
Standard error of the mean	23.9	20.3

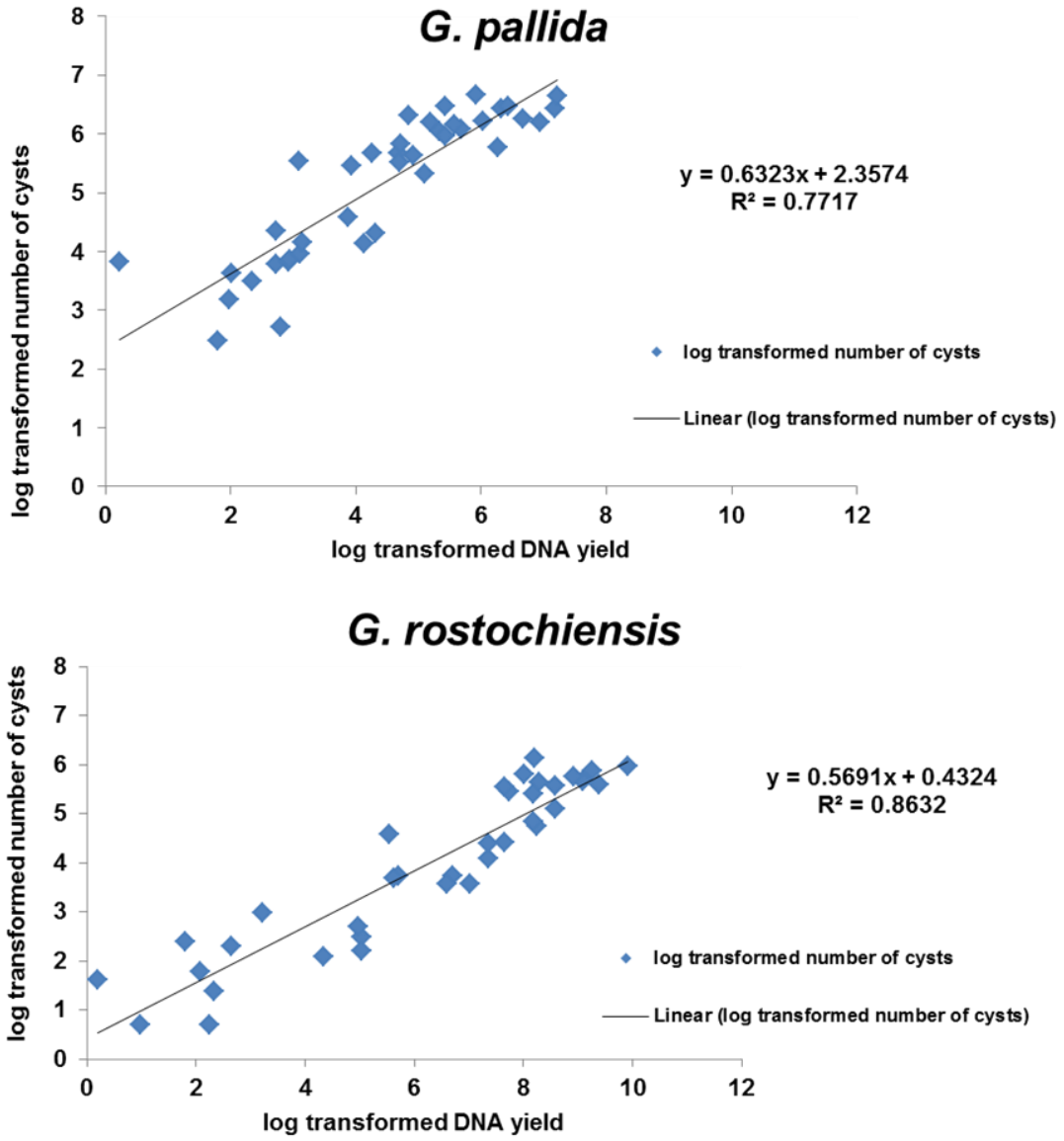


Figure 3.4 Correlation between DNA yield and numbers of cysts of *G.rostochiensis* and *G. pallida*.

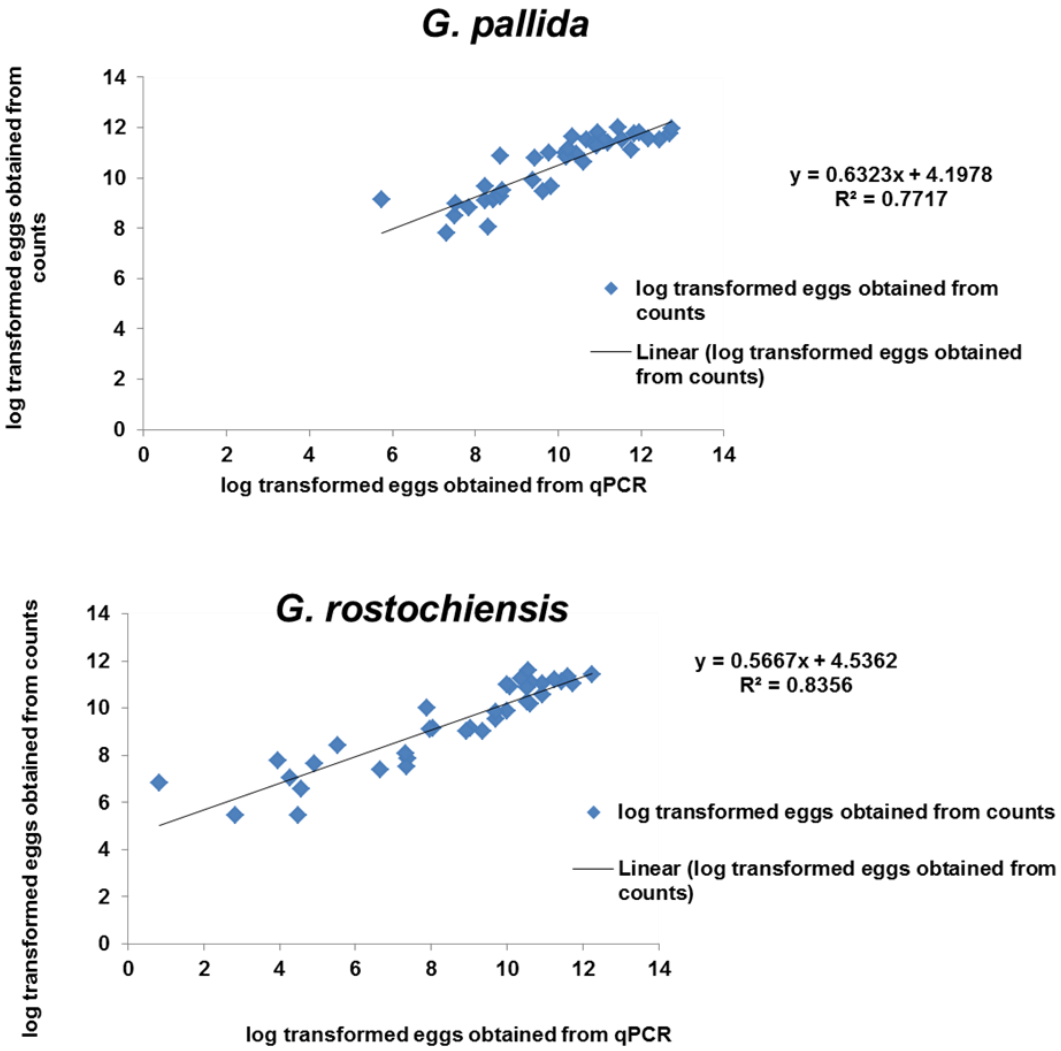


Figure 3.5 Relationship between the number of eggs of PCN obtained from counting and the resulting egg numbers determined by qPCR.

3.4.3. Interspecific competition experiment

3.4.3.1. Multiplication of *G. pallida* and *G. rostochiensis* as pure species and as mixtures

3.4.3.1.1. Numbers of cysts

The competition experiment showed an influence of the different genotypes of potato on PCN development. The highest numbers of cysts collected were, as expected, from susceptible cv Desirée, with both species and all inoculum compositions whereas cv Maris Piper significantly suppressed multiplication of PCN in the pots containing only inoculum of *G. rostochiensis*. However, in the pots with only *G. pallida,* there were no significant differences between cv Maris Piper and cv Desirée.

With inoculum containing 25% of *G. rostochiensis* and 75% of *G. pallida* the resulting composition with cvs Desirée and Maris Piper differed significantly from the reversed composition (75% of *G. rostochiensis* and 25% *G. pallida*). With cv Vales Everest multiplication of both species was significantly inhibited in comparison to cv Desirée. The cyst numbers with inoculum containing only *G. rostochiensis* showed greater multiplication with cv Vales Everest than with cv Maris Piper and significantly lower than with cv Desirée . Similarly with inoculum containing only *G. pallida* the cyst numbers with cv Vales Everest were significantly lower than with cv Maris Piper and cv Desirée.

ANOVA performed on the number of cysts with cultivar, and inoculum as factors confirmed a significant influence of the 3 cultivars on PCN multiplication

(P<0.001). Significant differences in cyst numbers between the different inoculum levels (P<0.001) were also observed.

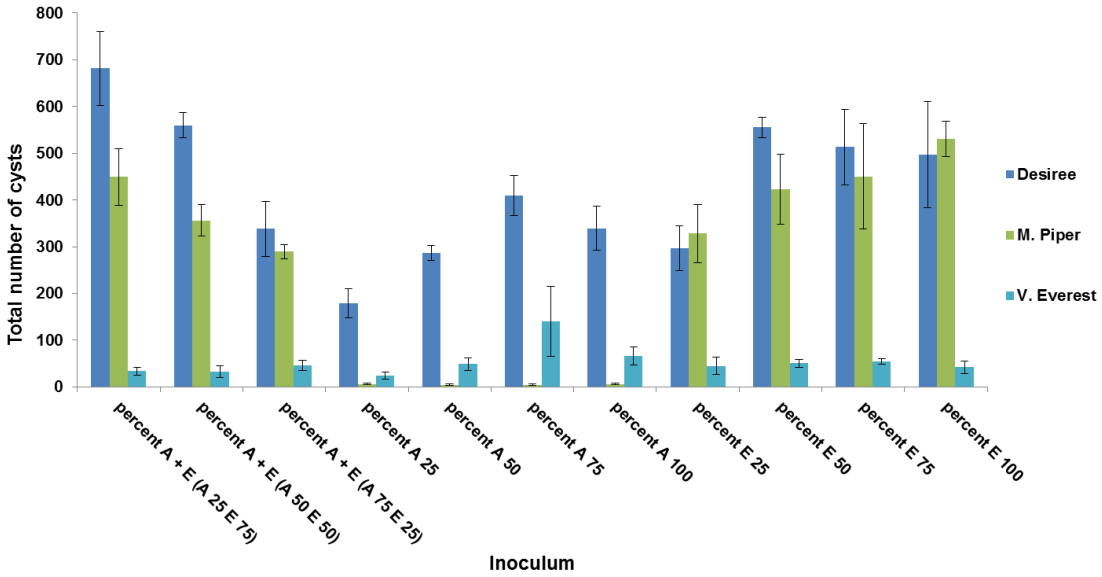


Figure 3.6 Total number of cysts of the 2 PCN species *G. pallida* (E) and *G. rostochiensis* (A) in the competition experiment at different inoculum densities (100% – 80 eggs/g, 75% – 60 eggs/g, 50% – 40 eggs/g and 25 – 20 eggs/g) and combinations (Table 3-1) in three different genotypes of potato a) cv Desirée, b) cv Maris Piper, c) cv Vales Everest. The bars are standard errors of the means for each cultivar.

3.4.3.2 Species composition determined using qPCR

The numbers of eggs/g soil determined by qPCR were square root transformed to normalize the data. Similar to the cyst results, the highest number of eggs/g soil was obtained from the susceptible cv Desirée. In order to demonstrate the variation between the inoculations and cultivars, the qPCR data were combined and are presented in Figure 3.6. The number of eggs/g soil of *G. pallida* with cv Desirée was relatively higher than on the cv Vales Everest. No differences were found in the number of eggs/g soil between different inoculums on the cv Desirée, similarly on cv Vales Everest the number of eggs/g soil of *G. pallida* did not differ between inoculums containing PCN. For the mixed inoculations, no difference in resulting composition was found with the different combinations except with 25% of *G. rostochiensis* and 75% of *G. pallida* on cvs Desirée and Vales Everest.

The multiplication rate of *G. rostochiensis* on cv Maris Piper was close to zero. On the cv Vales Everest there was significantly less multiplication of both species when the species were together than separately (Figure 3.7). Figure 3.8 presents the changes in the ratios between initial and final populations (Pf/Pi). The reproduction of both species of PCN on cv Vales Everest was reduced compared to on Desirée. The lowest multiplication rate (Pf/Pi) of *G. rostochiensis* was on cv Maris Piper in contrast to the susceptible Desirée. Also the multiplication rate was significantly higher in the low initial population densities with all cultivars compared to the results from pots with high numbers of eggs. A significant reduction in the multiplication rate of *G. rostochiensis* was found when *G. pallida* was present in higher or equal initial densities on the

susceptible cv Desirée, and a similar effect was observed on cv Vales Everest, however, when *G. rostochiensis* had a higher initial density, with mixed populations there was a significantly reduced reproduction rate of *G. pallida*.

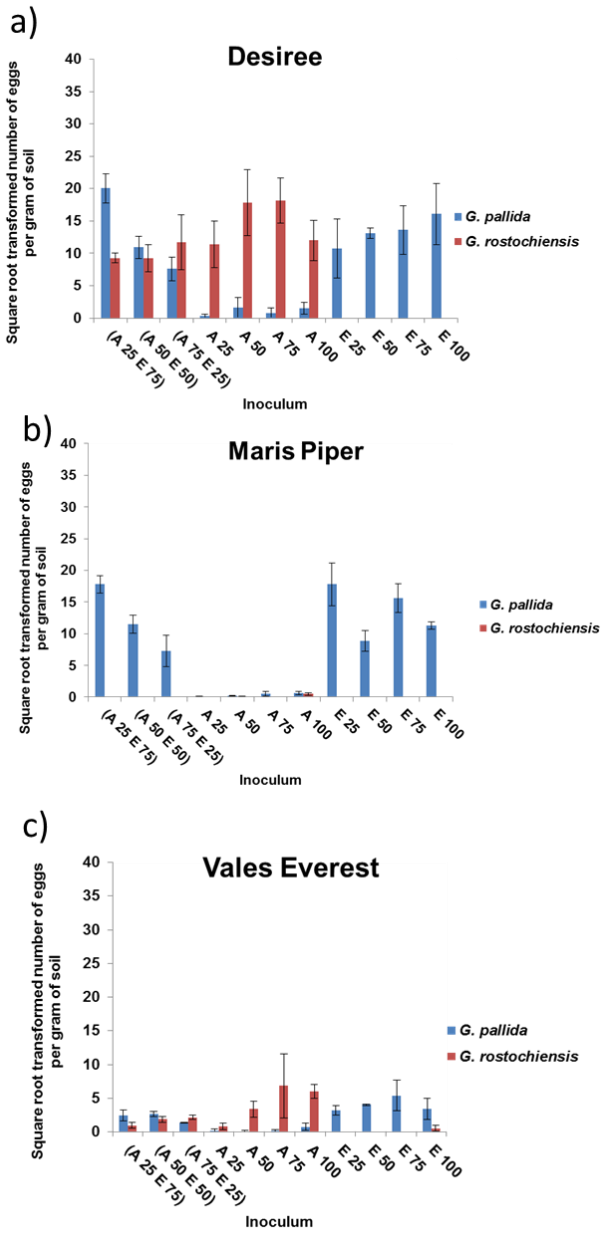


Figure 3.7 Square root transformed total number of eggs/g soil of the 2 PCN species *G. pallida* (E) and *G. rostochiensis* (A) in the competition experiment at different inoculum densities (100% – 80 eggs/g, 75% – 60 eggs/g, 50% – 40 eggs/g and 25 – 20 eggs/g) and combinations (Table 3-1) in three different genotypes of potato a) cv Desirée, b) cv Maris Piper, c) cv Vales Everest. The bars are standard errors of the means for each cultivar.

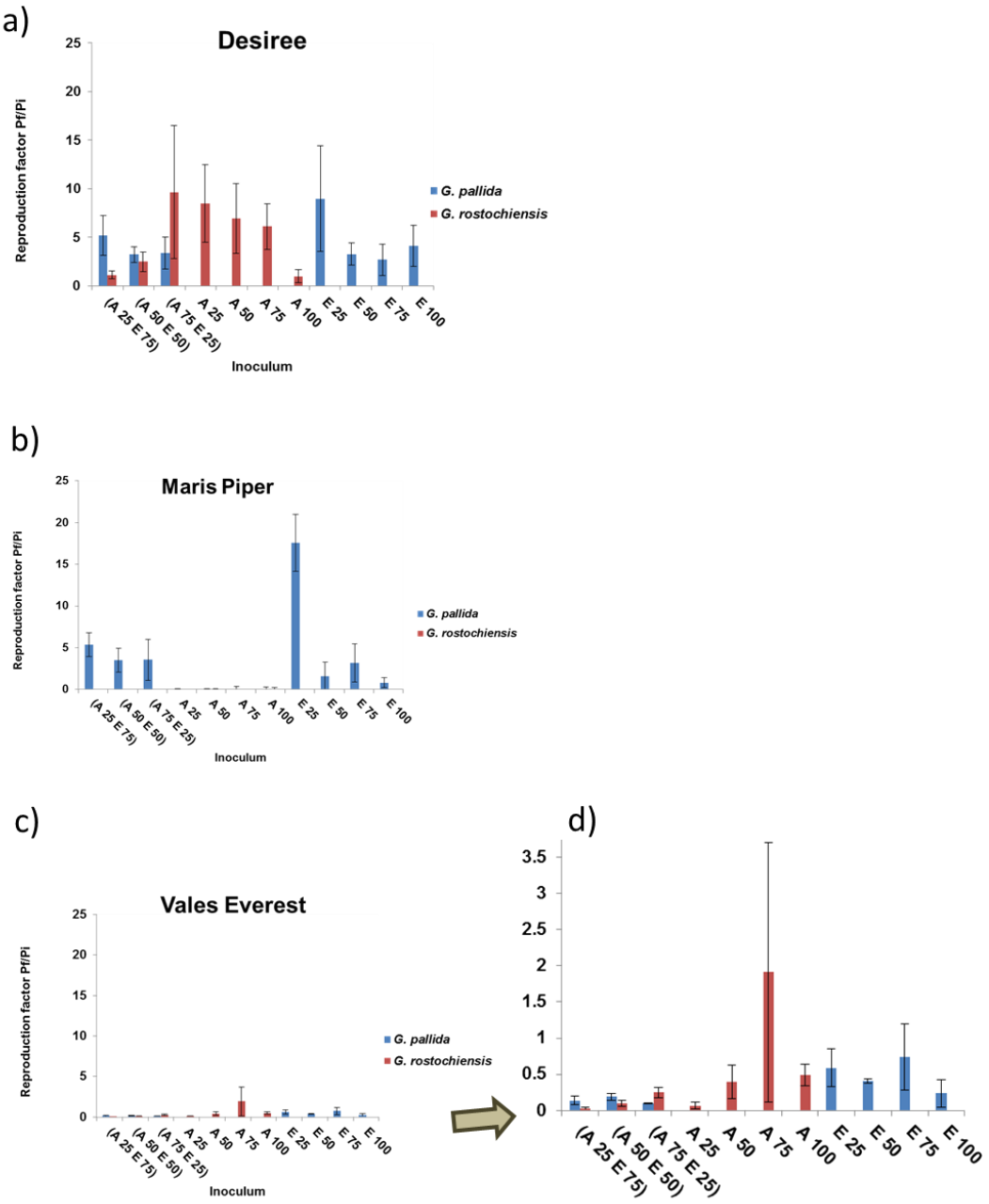


Figure 3.8 Reproduction factor (Pf/Pi ratio) after the 10 weeks of the 2 PCN species *G. pallida* (E) and *G. rostochiensis* (A) in the competition experiment at different inoculum densities (100% – 80 eggs/g, 75% – 60 eggs/g, 50% – 40 eggs/g and 25 – 20 eggs/g) and combinations (Table 3-1) in three different genotypes of potato a) cv Desirée, b) cv Maris Piper, c) cv Vales Everest d) magnification of the graph with cv Vales Everest. The bars are standard errors of the means for each cultivar.

3.4.4. Multiplication of PCN in the field experiments

The qPCR results were transformed using logarithmic (2011) and square roots (2012) transformations to normalize the data.

3.4.4.1. Luffness 2011 field trial

To estimate initial population levels (Pi), preplant soil samples were taken at the planting time and used to determine the eggs/g soil. Samples were tested by qPCR for both species of PCN. An average of 13.7 ± 1.8 (without nematicide plots) and 22.2 ± 2 (with nematicide plots) eggs/g soil of *G. pallida* were found. The qPCR showed no infestation of *G. rostochiensis.* The estimates of initial population levels showed an increasing trend in eggs/g soil in the infestation in the nematicide treated plots (Table 3-6).

Changes in the *G. pallida* populations over the growing season with and without nematicide treatment are presented in Figure 3.9 and Figure 3.10 respectively. In the plots without nematicide treatment, the numbers of eggs/g soil decreased slightly in harvest 1 which may be due to reduced egg content of the cysts following hatching. Between harvest 1 and 2 the number of eggs/g soil did not change and then decreased at harvest 3, 12 weeks after planting of the potatoes. After 16 weeks (harvest 4) the numbers of eggs/g soil increased rapidly to approximately twice the Pi. There was no significant difference in PCN multiplication between the cultivars (P=0.306). A similar trend was seen in the nematicide treated plots. In harvests 1, 2 and 3 the eggs/g soil were lower than in the preplant samples but after 16 weeks (harvest 4) the number of eggs/g soil increased in 3 cultivars, Cara, Estima and Maris Piper, whereas with cv Desirée

it remained at the same level as harvests 1, 2 and 3. There were no significant differences between cultivars in the final levels of infestation with either treatments (P=0.69), however the final population levels were significantly lower with the nematicide treatment (P<0.001). Moreover the interaction between the treatments (with or without nematicide) and time of harvesting was significant (P<0.001).

In order to demonstrate the variation between treatments over the growing season, the results from untreated and nematicide treated plots were combined and are presented in Figure 3.11. The relative increase in the number of eggs/g was clearly visible without nematicide treatment at harvests 4 and 5 in contrast to the nematicide treatment which showed a gradual rise in eggs/g soil during the growing season but did not become as high as the without nematicide treatment. To assess the influence of with and without nematicide treatment and different cultivars on multiplication (Pf/Pi) (Figure 3.12), an ANOVA was used. It revealed no significant differences in the reproduction factor between the cultivars (P=0.463), however there was a significant influence in whether or not there was a nematicide treatment on population multiplication (P<0.001).

Table 3-6 Layout of the field trial at Luffness 2011 showing the initial populations of *G. pallida* within the field in eggs per gram of soil at the 6 different harvest areas determined by qPCR. Two samples from Harvests 3 and 4 were lost during the qPCR analysis.

Nematicide	No nematicide	Nematicide	No nematicide
H1	H1	H2	H2
9.38	6.85	4.65	12.55
12.77	2.13	12.27	6.65
9.94	10.39	5.83	22.48
13.60	10.13	10.07	9.99
12.67	9.63	9.84	5.82

H3	H3	H4	H4
11.37		11.35	0.46
10.06	5.60	28.65	9.28
12.00	5.48	35.14	4.32
12.12	2.72	34.47	
9.51	12.62	37.24	1.43

H5	H5	H6	H6
25.30	5.33	29.33	15.84
20.21	5.60	46.50	20.08
23.69	21.38	20.25	13.47
28.72	11.50	38.92	16.82
26.23	33.30	42.54	32.95

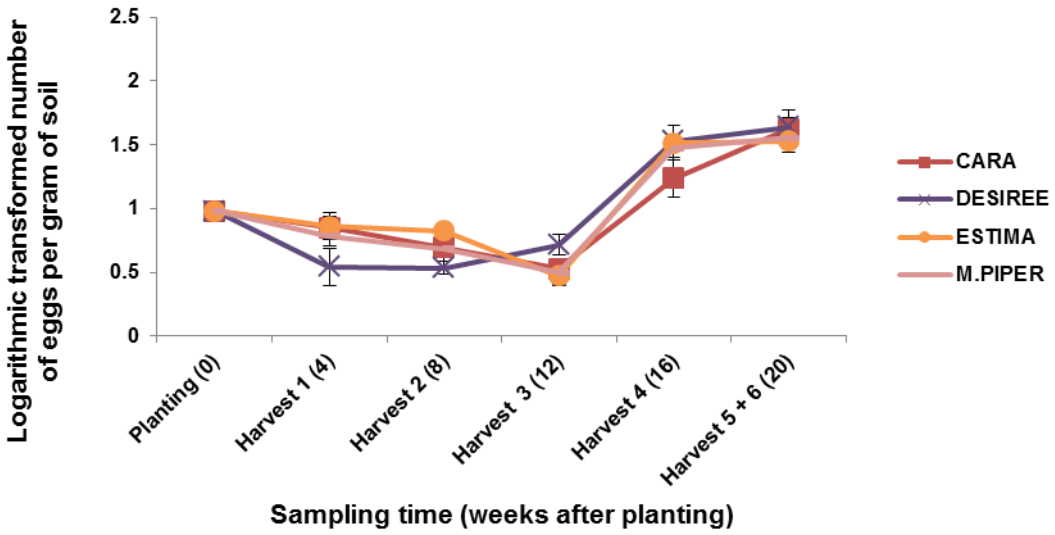


Figure 3.9 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season without using nematicides at Luffness in 2011. The bars are standard errors of the means for each cultivar.

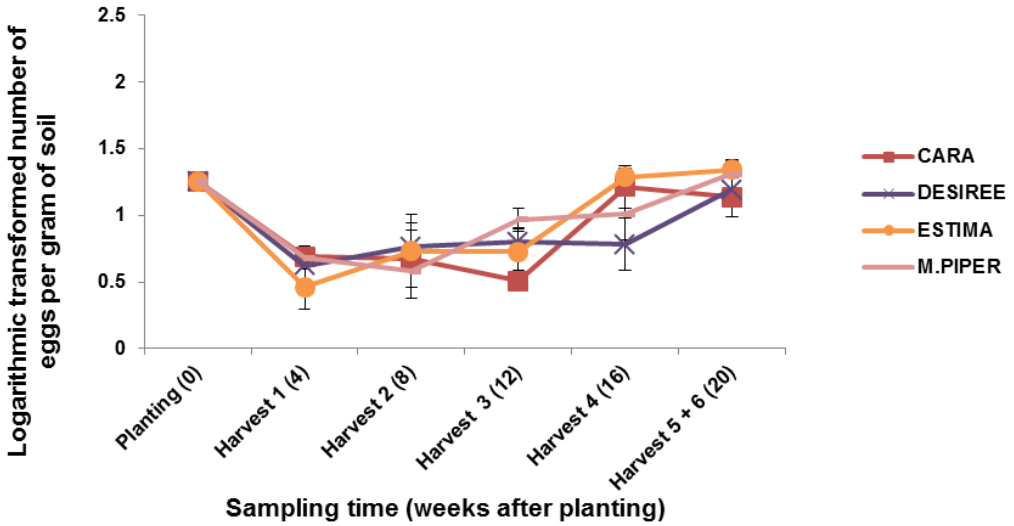


Figure 3.10 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season with nematicides at Luffness in 2011. The bars are standard errors of the means for each cultivar.

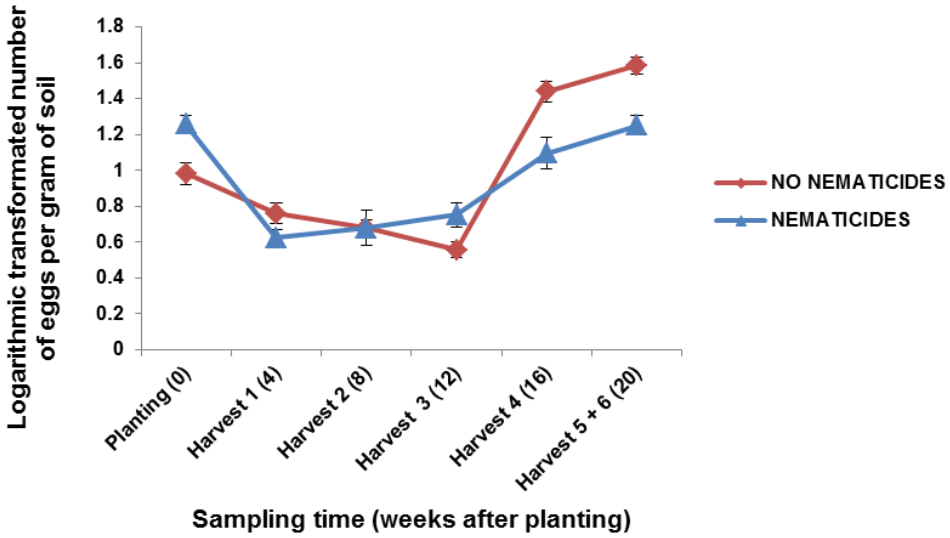


Figure 3.11 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season at Luffness in 2011 from untreated and nematicide treated plots over the 20 week growing period. The bars are standard errors of the means of four cultivars used for this study.

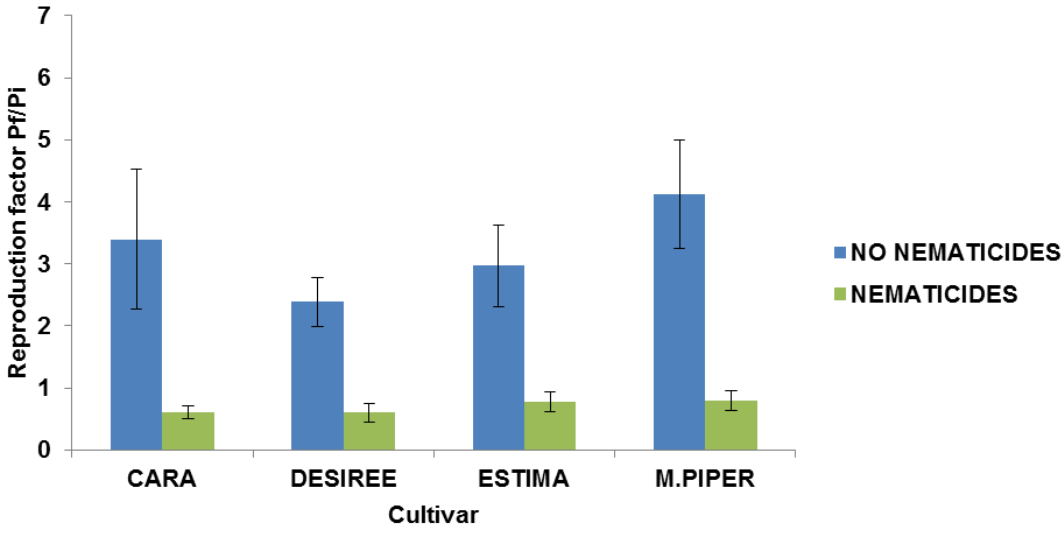


Figure 3.12 Reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil determined by qPCR after the 20 weeks of planting. The bars are standard errors of the means for each cultivar.

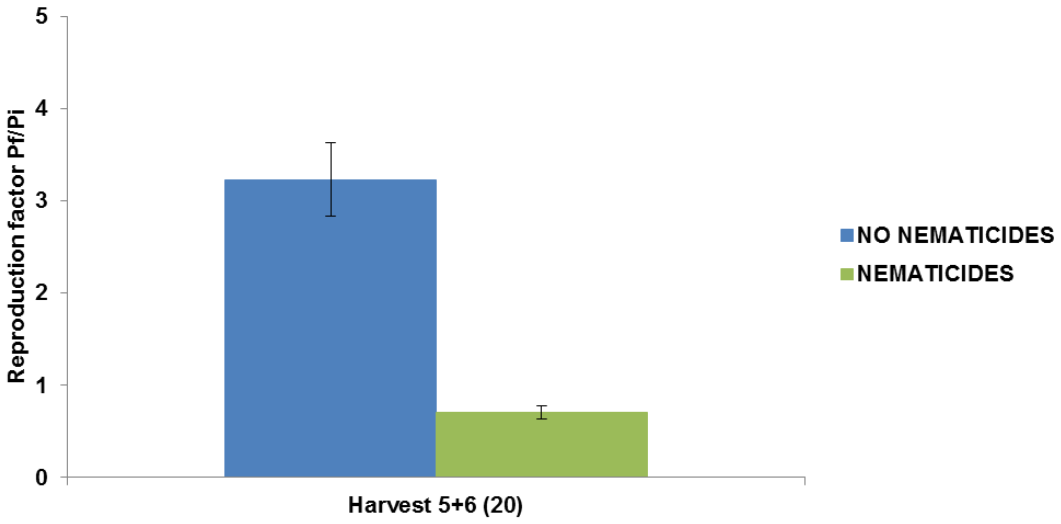


Figure 3.13 Average reproduction factor (Pf/Pi ratio) estimated with the number of eggs/g soil determined qPCR after the 20 weeks of planting. The bars are standard errors of the means of four cultivars used for this study.

3.4.4.2. Luffness 2012 field trial

To estimate the initial population, preplanting soil samples were taken at planting time in May. An average of 0.08 ± 0.03 (non-nematicide plots) and 0.06 ± 0.02 (nematicide treatment) eggs per gram of soil were found in the experiment plots at the beginning of the experiment. Table 3-7 shows the level of initial infestation of *G. pallida* in the field as eggs per gram of soil. All the samples were tested for *G. rostochiensis* and only the field with no nematicide treatment showed a small initial infestation of this species, with an average 0.03 ± 0.02 eggs per gram of soil.

Changes in the populations over the growing season in the plots without nematicides are presented in Figure 3.14. The numbers of eggs/g soil decreased slightly 4 weeks after planting at the end of May. This drop was noted in all 4 cultivars and is consistent with the reduction in egg content of the cysts following hatching. Between weeks 4 and 12 the quantity of eggs in the soil remained the same. In September, 16 weeks after planting, all the cultivars showed an increase in the number of eggs per gram soil. The highest peak was recorded for cv Cara, the second highest was with Maris Piper. At the end of October in the final harvest the highest eggs/g soil was recorded with cv Desirée.

Fluctuations in the population levels over the growing season in the plots without nematicides are presented on the Figure 3.15. Similar to the non-treated

plots, the numbers of eggs decreased within the 12 weeks after planting potatoes. In September, 16 weeks after planting, the number of eggs in the soil increased with cvs Desirée, Maris Piper and Cara, while with cv Vales Everest they stayed at the same level. After 22 weeks from planting, the population was still rising on cv Maris Piper, however they decreased with cvs Desirée and Cara. With cv Vales Everest the eggs/g soil remained at a low level throughout the trial.

A comparison of the combined data for the 4 cultivars is shown on the Figure 3.16. The amount of *G. pallida* detected from the plots treated with nematicide demonstrated the same pattern as from the non-treated plots. Both decreased within the first 8 weeks and rose slightly at week 12. The biggest difference was apparent between weeks 12 and 22. In September, the population rapidly increased and continued rising till October. Figure 3.17 and Figure 3.18 show how the ratio between initial and final population changed over the 22 weeks of the trial. In both treatments the final population has risen. Due to the low initial eggs/g soil, the average of the initial samples per experimental field was established in order to compare the multiplication rate on different cultivars as well as with or without nematicide.

Table 3-7 Layout of the field trial at Luffness 2012 showing the initial populations of *G. pallida* within the field in eggs per gram of soil at the 6 different harvest areas determined by qPCR. One sample from Harvests 3 was lost during the qPCR analysis.

No nematicide		Nematicide	
H1	H2	H1	H2
0.004	0.007	0.198	0.001
0.005	0.001	0.000	0.418
0.203	0.000	0.136	0.000
0.015	0.274	0.000	0.003
0.000	0.049	0.000	0.000
H3	H4	H3	H4
0.000	0.000	0.000	0.000
0.029	0.010		0.002
0.004	0.366	0.564	0.031
0.017	0.172	0.001	0.213
0.366	0.020	0.018	0.010
H5	H6	H5	H6
0.026	0.000	0.004	0.000
0.025	0.905	0.000	0.000
0.000	0.012	0.002	0.000
0.010	0.003	0.003	0.214
0.009	0.000	0.000	0.000

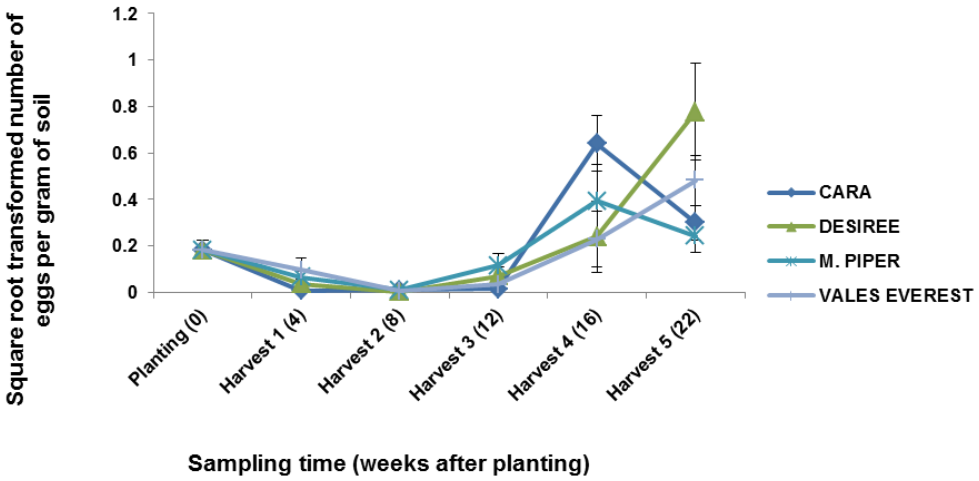


Figure 3.14 Square root transformed number of eggs/g soil determined by qPCR over the growing season without using nematicides at Luffness in 2012. The bars are standard errors of the means for each cultivar.

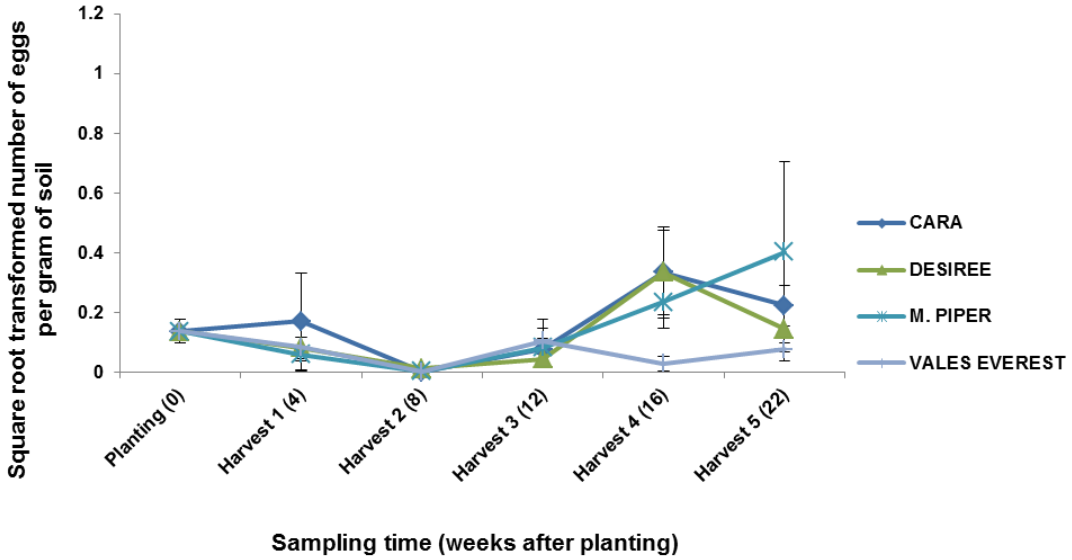


Figure 3.15 Square root transformed number of eggs/g soil determined by qPCR over the growing season with nematicides at Luffness in 2012. The bars are standard errors of the means for each cultivar.

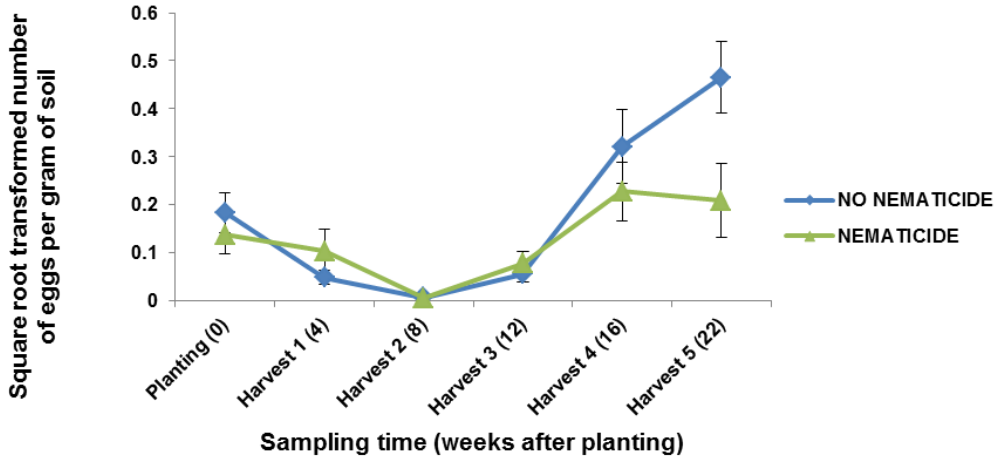


Figure 3.16 Square root transformed number of eggs/g determined by qPCR over the growing season at Luffness in 2012 from untreated and nematicide treated plots over the 20 week growing period. The bars are standard errors of the means of four cultivars used for this study.

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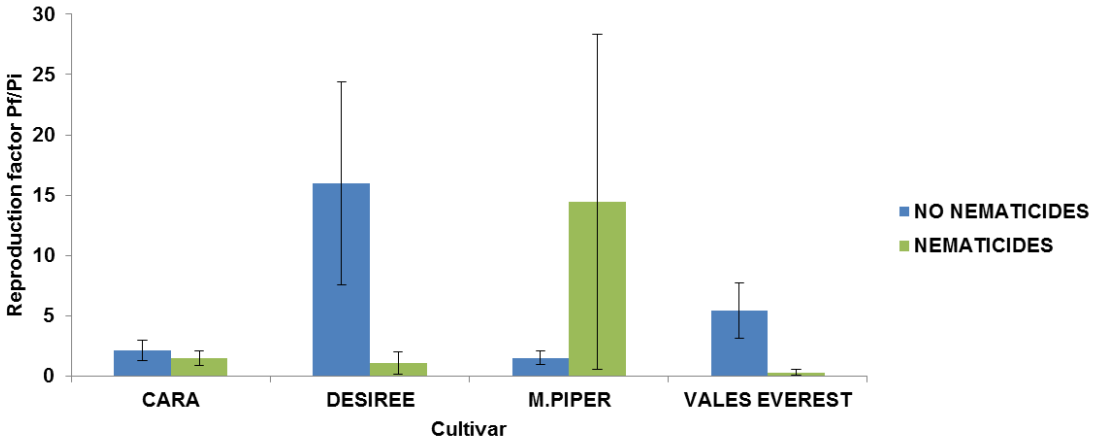

Figure 3.17 Reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil determined by qPCR after the 24 weeks of planting. The bars are standard errors of the means for each cultivar.

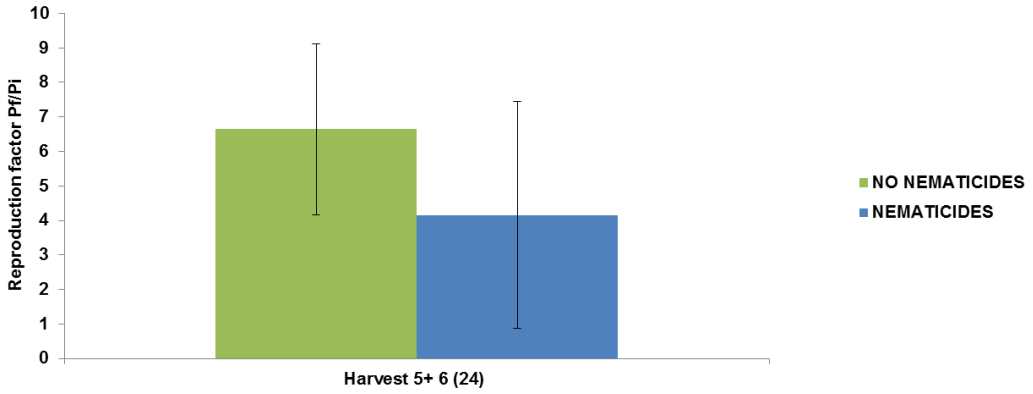

Figure 3.18 Reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil determined by qPCR after the 24 weeks of planting. The bars are standard errors of the means of four cultivars used for this study.

3.4.4.3. Harper Adams 2011 field trial

To estimate the initial population level of *G. pallida* in the plots for the Harper Adams field trial in 2011, preplant samples were taken at planting in April. The Harper Adams 2011 preplant samples had an average of 9.7± 0.6 (without nematicide) and 6.2 ± 0.9 (with nematicide) eggs/g soil. Samples were negative when tested by qPCR for *G. rostochiensis.*

Changes in the eggs/g soil over the growing season in the plots without nematicide are presented in Figure 3.19. Four and 8 weeks after planting (harvests 1 and 2) the number of eggs/g soil had decreased compared to the preplant levels. In August at harvest 4 (16 weeks), all of the cultivars showed a significant increase in the eggs/g soil and for all cultivars the final population levels were higher than the initial Pi. However there was no significant difference between cultivars in response to PCN (P=0.992). The means of the eggs/g for the 4 cultivars per harvest with and without treatment were established and plotted in the Figure 3.21. There was a significant difference in eggs/g of soil between treated and untreated plots (P<0.001) over the growing season.

The final population (Pf) was significantly higher than the initial population (Pi) with a mean egg/g of soil of 50 ± 0.6 in the untreated and 21.8 ± 0.5 in treated plots. The reproduction factor Pf/Pi increased to 3.19 ± 0.56 and 2.50 ± 0.46 overall in the untreated and treated plots respectively. To assess the influence of nematicides and different cultivars on a multiplication an ANOVA was used. No significant differences were found in a reproduction factor between the

cultivars (P=0.305) or nematicide effect on a population multiplication rates (P=0.409).

Table 3-8 Layout of the field trial at Harper Adams 2011 showing the initial populations of *G. pallida* within the field in eggs per gram of soil at the 6 different harvest areas determined by qPCR. Two samples from Harvest 3 and three from Harvest 5 were lost during the qPCR analysis.

No nematicide	No nematicide	Nematicide	Nematicide
H1	H2	H1	H2
13.51	8.15	0.88	3.08
3.72	8.64	9.41	7.81
8.73	9.62	0.03	8.28
10.74	8.00	10.49	9.54
11.21	6.50	0.17	3.91
H3	H4	H3	H4
4.33	9.29		9.37
7.13	14.28	8.99	4.31
13.88	10.67	1.06	7.29
9.56	9.13		11.81
15.64	9.86	0.05	0.34
H5	H6	H5	H6
5.99	7.24	11.26	4.17
3.22	16.68		12.78
8.76	10.58		5.44
13.24	12.70		11.52
13.04	6.51	0.03	13.29

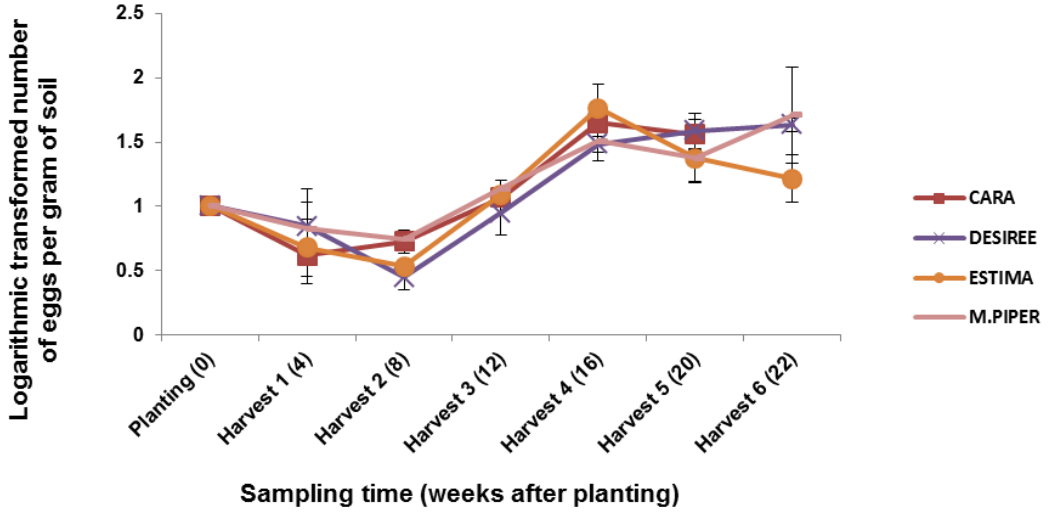

Figure 3.19 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season without using nematicides at Harper Adams in 2011. The bars are standard errors of the means for each cultivar.

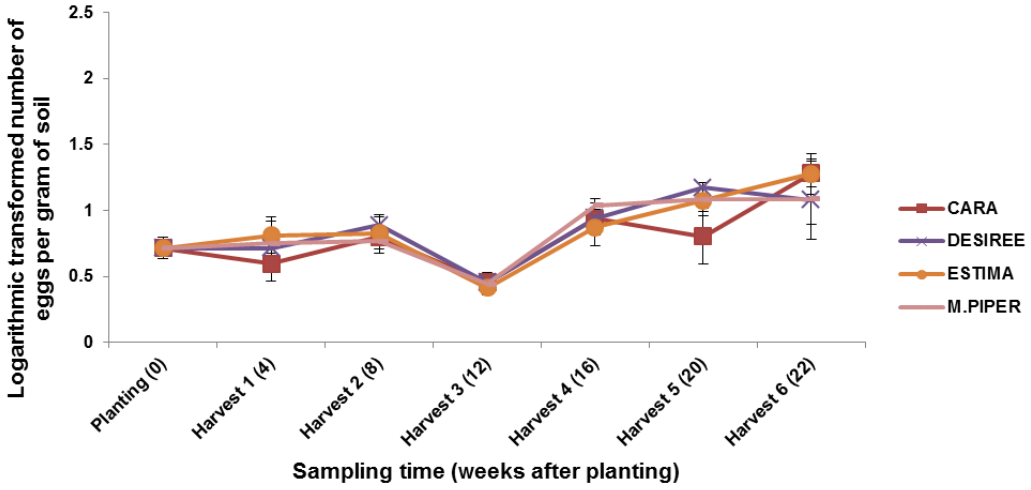

Figure 3.20 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season with using nematicides at Harper Adams in 2011. The bars are standard errors of the means for each cultivar.

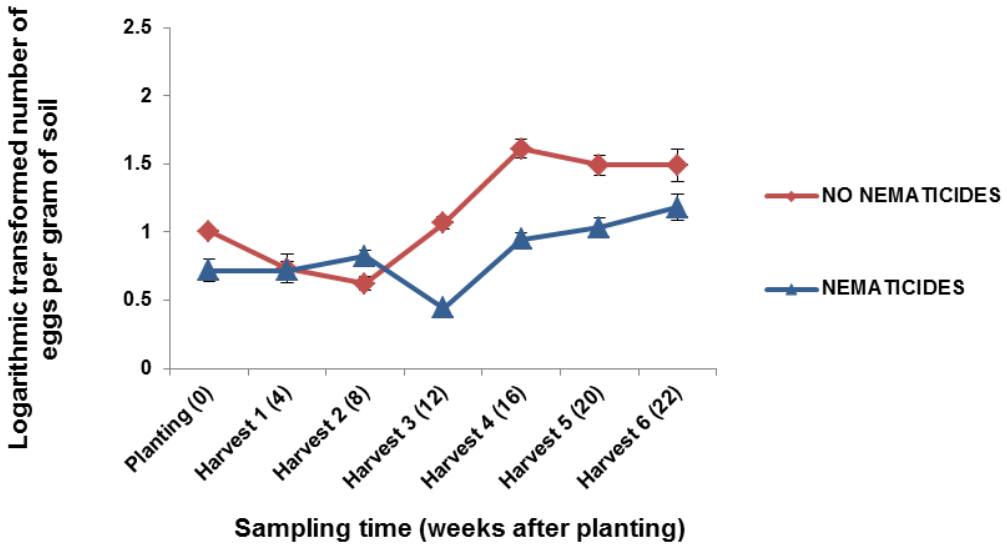

Figure 3.21 Logarithmic transformed number of eggs/g soil determined by qPCR over the growing season at Harper Adams in 2011 from untreated and nematicide treated plots over the 22 week growing period The bars are standard errors of the means of four cultivars used for this study.

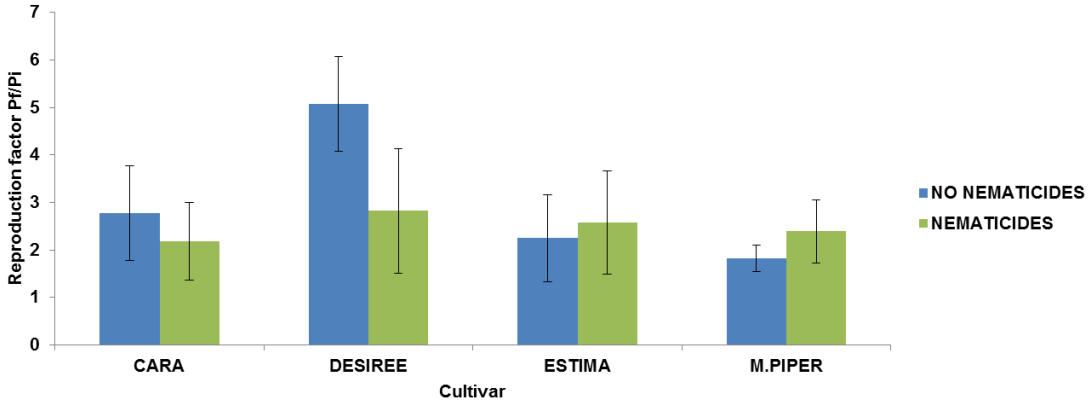

Figure 3.22 Reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil determined by qPCR after the 20 weeks of planting. The bars are standard errors of the means for the each cultivar.

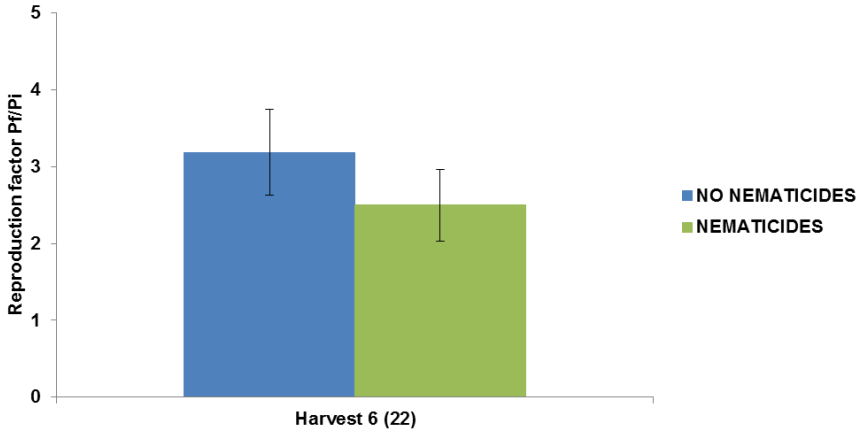

Figure 3.23 Average reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil determined by qPCR after the 22 weeks of planting. The bars are standard errors of the means of four cultivars used for this study.

3.4.4.4. Harper Adams 2012 field trial

To estimate the initial population, preplant samples were taken at planting time in April. An average of 0.08 ± 0.026 (non-nematicide plots) and 0.15 ± 0.08 (nematicide treatment) eggs/gram soil were found in the plots at the beginning of the experiment. Table 3-9 shows the level of infestation of *G. pallida* in the plots.

Figure 3.24 shows changes in the population of *G. pallida* in the field plots without the application of nematicide. Over the first 12 weeks after planting, the population was not changed. In August, 16 weeks after planting, the quantity of eggs in the soil increased for all cultivars. After 20 weeks, the number of eggs in the soil dropped in cvs M. Piper and Vales Everest. In the plots with cvs Desirée and Cara the number of eggs continued rising until the last harvest at the beginning of October. In the final harvest, Desirée had the largest number of eggs/g soil while Vales Everest exerted the best control, with the lowest number of eggs/g soil. In the nematicide treated field (Figure 3.25), the infestation stayed at the same level, similar to the untreated plots over first 12 weeks of growing potatoes. For all of the cultivars, the peak quantity of eggs was observed after 16 weeks. The highest number of eggs was recorded on the susceptible Desirée. In September, all cultivars showed a decrease in the number of eggs with only the cv Cara population continuing to rise. At the end of the field trial the number of eggs increased slightly, the highest peak of PCN in the soil was recorded on the cv Cara and lowest in Vales Everest. In Figure

3.26 a comparison of treated and non treated plots is shown as a mean of all 4 cultivars. It is apparent that the pattern of population changes was the same for both treatments. There was an increase of number of eggs per gram of soil in August, then a drop in September and again an increase in October. Similar to Luffness, due to the initially low number of eggs/g soil, the average of initial samples was determined in order to compare the multiplication rate on the different cultivars as well as with or without nematicides.

Table 3-9 Layout of the field trial at Harper Adams 2012 showing the initial populations of *G. pallida* within the field in eggs per gram of soil at the 6 different harvest areas determined by qPCR. One sample from Harvest 5 was lost during the qPCR analysis.

No nematicide		Nematicide	
H1	H2	H1	H2
0.008	0.467	0.000	0.001
0.000	0.005	0.000	0.614
0.031	0.100	0.000	0.001
0.028	0.038	0.028	0.042
0.098	0.640	0.000	0.000
H3	H4	H3	H4
0.148	0.013	0.001	2.117
0.000	0.061	0.000	0.000
0.002	0.075	0.091	0.002
0.001	0.037	0.000	0.000
0.077	0.151	1.416	0.026
H5	H6	H5	H6
0.000	0.000	0.000	0.000
0.002	0.005	0.000	0.014
0.277	0.001	0.001	0.000
0.008	0.000	0.044	0.001
0.168	0.007		0.042

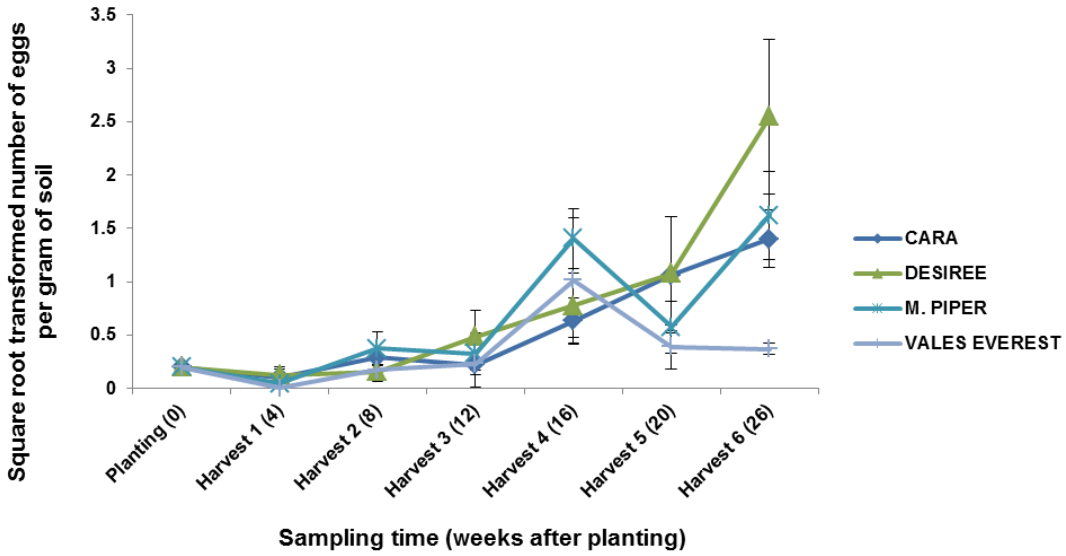

Figure 3.24 Square root transformed number of eggs/g soil obtained from qPCR over the growing season without using nematicides at Harper Adams in 2012. The bars are standard errors of the means for each cultivar.

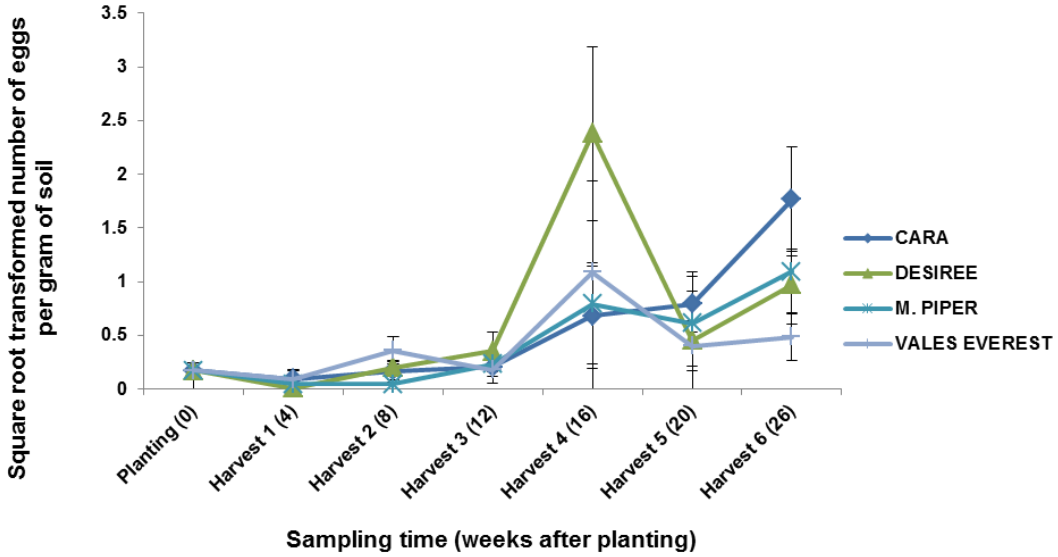

Figure 3.25 Square root transformed number of eggs/g soil obtained from qPCR over the growing season with nematicides at Harper Adams in 2012. The bars are standard errors of the means for each cultivar.

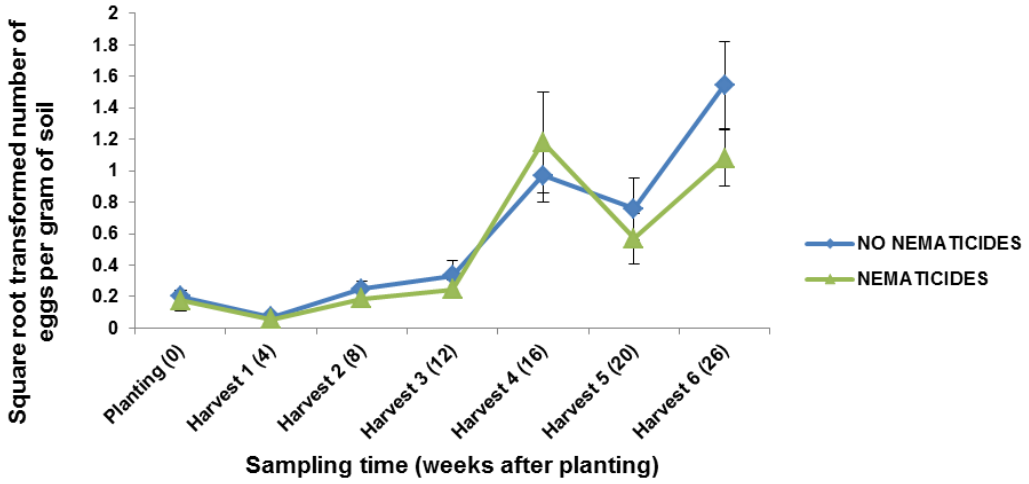

Figure 3.26 Logarithmic transformed number of eggs/g soil obtained from qPCR over the growing season at Harper Adams in 2012 from untreated and nematicide treated plots over the 26 week growing period. The bars are standard errors of the means of four cultivars used for this study.

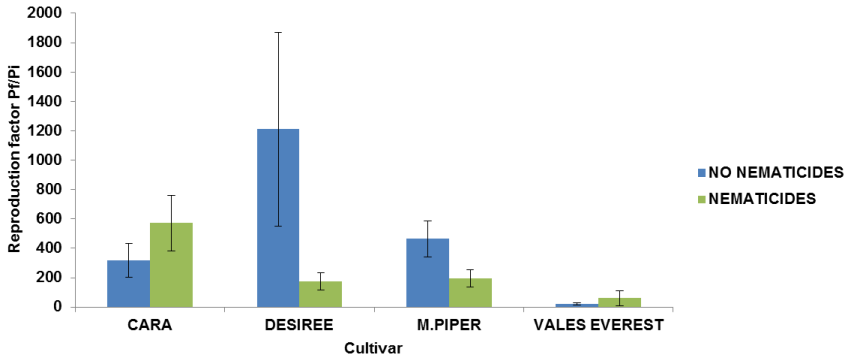

Figure 3.27 Reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil obtained from qPCR 26 weeks after planting. The bars are standard errors of the means for the each cultivar.

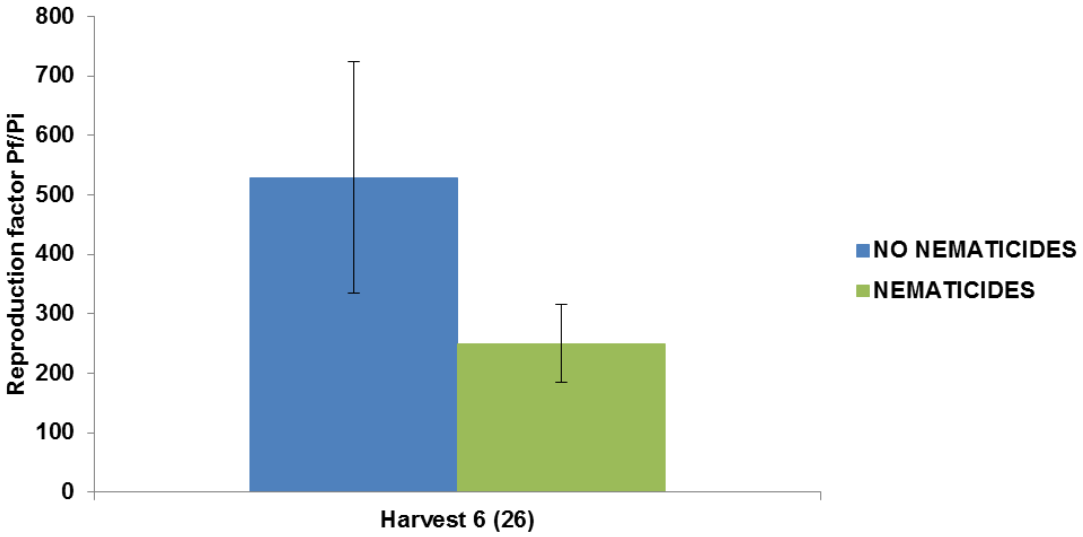

Figure 3.28 Average reproduction factor (Pf/Pi ratio) estimated with number of eggs/g soil obtained from qPCR 26 weeks after planting. The bars are standard errors of the means of four cultivars used for this study.

3.4.4.5. Relationship between initial population and multiplication rate (Pf/Pi)

Due to very low infestation in the experimental plots in both Luffness and Harper Adams in 2012, the relationship between initial population and multiplication rate was investigated only from data obtained in the year 2011.

According to the scale presented by Trudgill *et al.,* (2014), the sites chosen for the field experiments in 2011 had broad levels of infestation and the Pi ranged from low to high. The Harper Adams site was lightly to moderately infested with *G. pallida* and the site in Luffness varied from lightly to heavily infested with a range in the initial population densities (<1 to > 45 eggs/g soil). The results indicate that in both sites, in the untreated plots there was a clear trend towards decreasing multiplication rate with increase in the initial population (Figure 3.29 and Figure 3.30).

At Luffness the multiplication rate was significantly higher in the plots with lower initial densities ($P<0.001$). Also, as shown previously, the Pf/Pi was clearly reduced by the nematicide as the Vydate treatment significantly reduced multiplication in heavily infested plots.

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 different sites ($P=0.028$) but there was no difference in multiplication rate associated with different cultivars ($P=0.835$).

In the Harper Adams plots, the nematicide application did not show any trend in the Pi and Pf/Pi and the results were variable with no significant difference ($P= 0.222$).

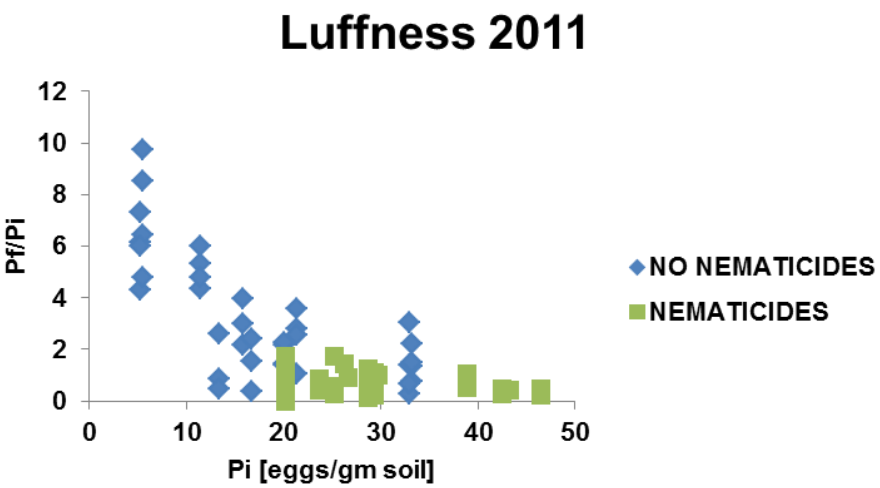

Figure 3.29 Relationship between multiplication rate and initial density at Luffness in 2011. Reproduction factor (Pf/Pi ratio) is plotted against the population density at planting (Pi) in untreated (blue) and nematicide treated (green) experimental plots.

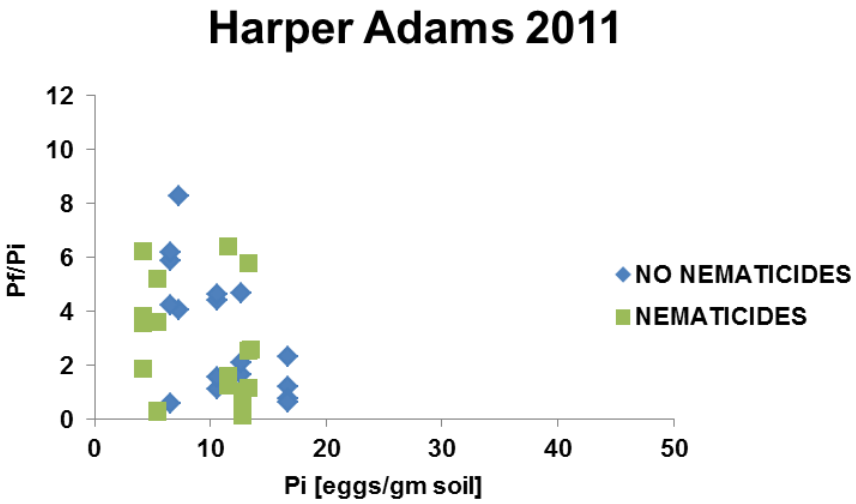

Figure 3.30 Relationship between multiplication rate and initial density at Harper Adams in 2011. Reproduction factor (Pf/Pi ratio) is plotted against the population density at planting (Pi) in untreated (blue) and nematicide treated (green) experimental plots.

3.4.4.6. Relationship between soil temperatures and multiplication of PCN in the field conditions

In 2011, the mean soil temperature over 150 days of the growing season at Luffness site was 14.15°C and at Harper Adams 14.85°C. In 2012 at Harper Adams site was 13.72°C over 186 days of the growing season (Figure 3.31, Figure 3.32 and Figure 3.33). Due to the damage of the thermochrones, temperatures from Luffness in 2012 could not be obtained; therefore comparing the temperature profiles between two sites in 2012 and the PCN response to temperature was not possible. A t-test performed on data from both experimental fields in 2011 and one in 2012 confirmed that there were significant differences between the sites ($P<0.001$) in the soil temperature. The soil temperatures were also significantly higher at Harper Adams than in Luffness at the latter part of the growing season.

Figure 3.33 and Figure 3.34 present combined results from field experiments performed in 2011, details of the achieved results are presented in sections 3.4.1., 3.4.4.1 and 3.4.4.4.

Results determined by qPCR showed that in both sites, at the first harvest there was a reduction of number of eggs/g soil compared to initial population. Visual analysis of stained roots from harvest 1 confirmed that within the first 4 weeks, hatching occurred and the juveniles invaded roots with the mean soil temperature between planting and first harvest 13.88 ± 0.18°C at the Scottish site compared to 14.38 ±10.39°C at the English site (Figure 3.33 and Figure 3.34). Different stages of juveniles (section 3.4.4.1.) were recorded until harvest 3 (12 weeks). By visual analysis females were recorded at both experimental

sites at harvests 2 and 3, after which the increase of eggs/g soil obtained by qPCR was observed, suggesting that females achieved maturity, detached from roots and consequently become a cysts. The mean soil temperatures recorded between harvest 2 and harvest 3 were at Harper Adams 13.91 ± 0.28°C and at Luffness 14.96 ± 0.20°C.

At Harper Adams the temperature increased to 15.64 ± 0.33°C between harvest 4 and 5 (16-20 weeks) and harvest 4 and 5 16.25 ± 0.26°C (between 20-22 weeks) in contrast to the Scottish Luffness site where the soil temperatures dropped to 13.64 ± 0.17°C (between 16-20 weeks). Interestingly, at harvest 5 (week 20) and harvest 6 (22 weeks) juveniles as well as females were detected inside the stained roots sampled at the Harper Adams site, which might suggest that a second hatch may have occurred. The second invasion was not observed inside roots at Luffness, however it is difficult to decide whether that was an effect of lower temperatures or the lack of the suitable host as the potato plants showed symptoms of death in both treatments and cultivars.

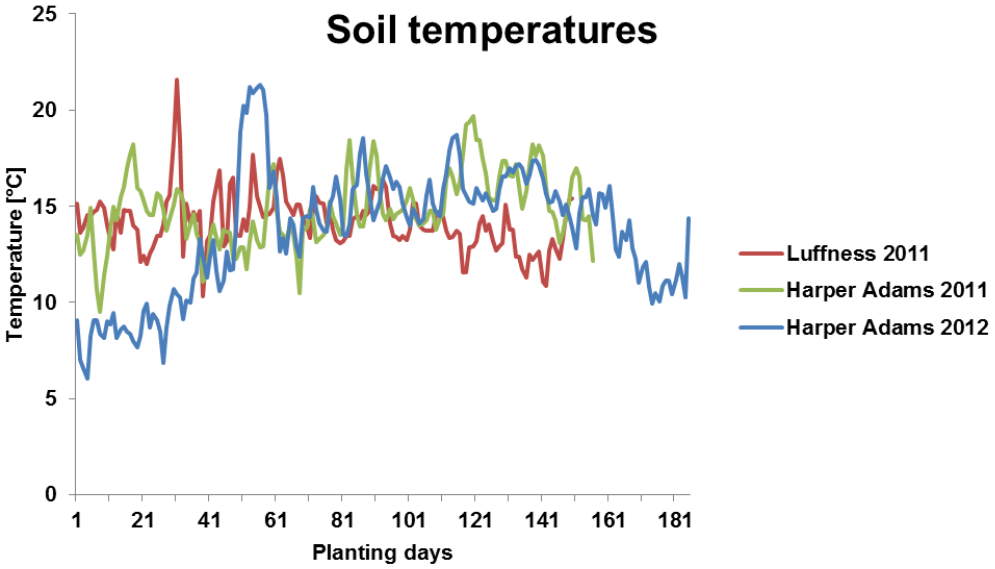

Figure 3.31 Soil temperatures over growing season in Harper Adams and Luffness in 2011 and in Harper Adams in 2012.

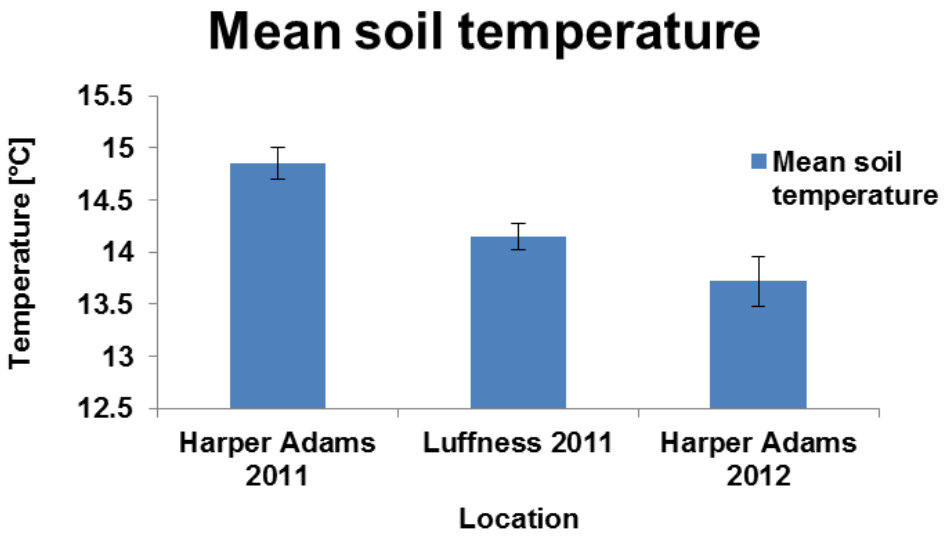

Figure 3.32 Mean soil temperature over the growing season in experimental sites.

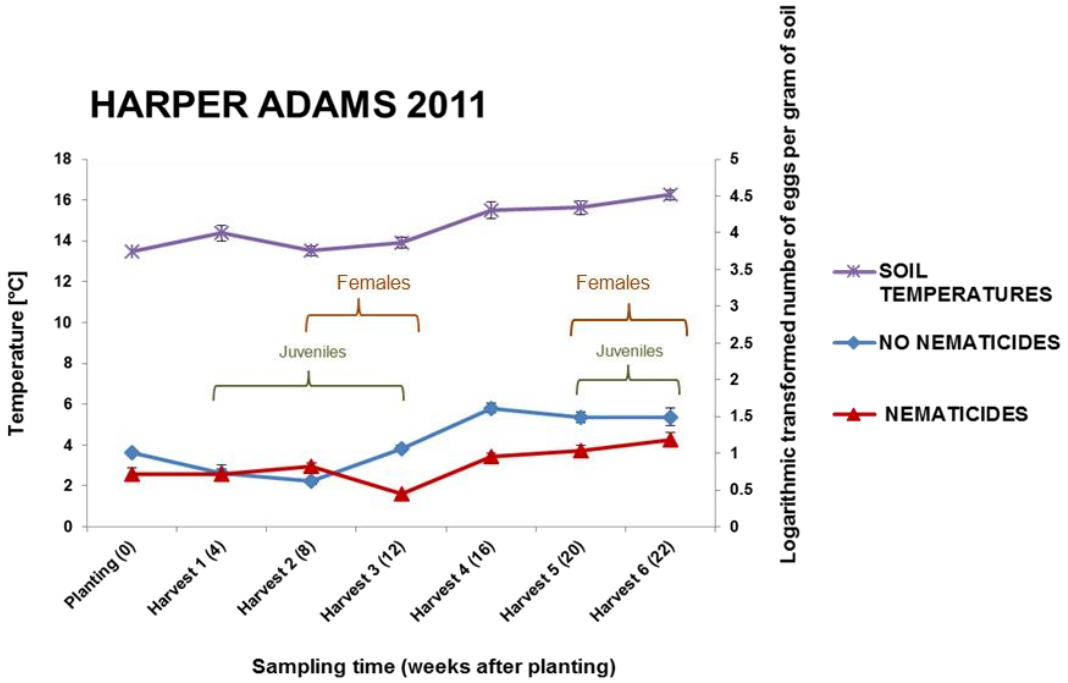

Figure 3.33 Comparison of the different developmental stages of PCN determined by qPCR and observation inside acid fuchsin stained roots of cultivars Cara and Desirée at Harper Adams in 2011 field trial. Primary y axis is the mean temperatures between the harvests. Secondary y axis is the logarithmic transformed number of eggs/g soil determined by qPCR over the growing season from untreated and nematicide treated plots over the 22 week growing period. Juveniles stages (J2, J3, J4) were combined and are presented as "Juveniles". Root samples were examined at harvests 1, 2, 3, 4, 5 and 6 (weeks 4, 8, 12, 16, 20 and 22). The bars are standard errors of the means of four cultivars used for this study and mean of temperatures per 4 week period between each harvest.

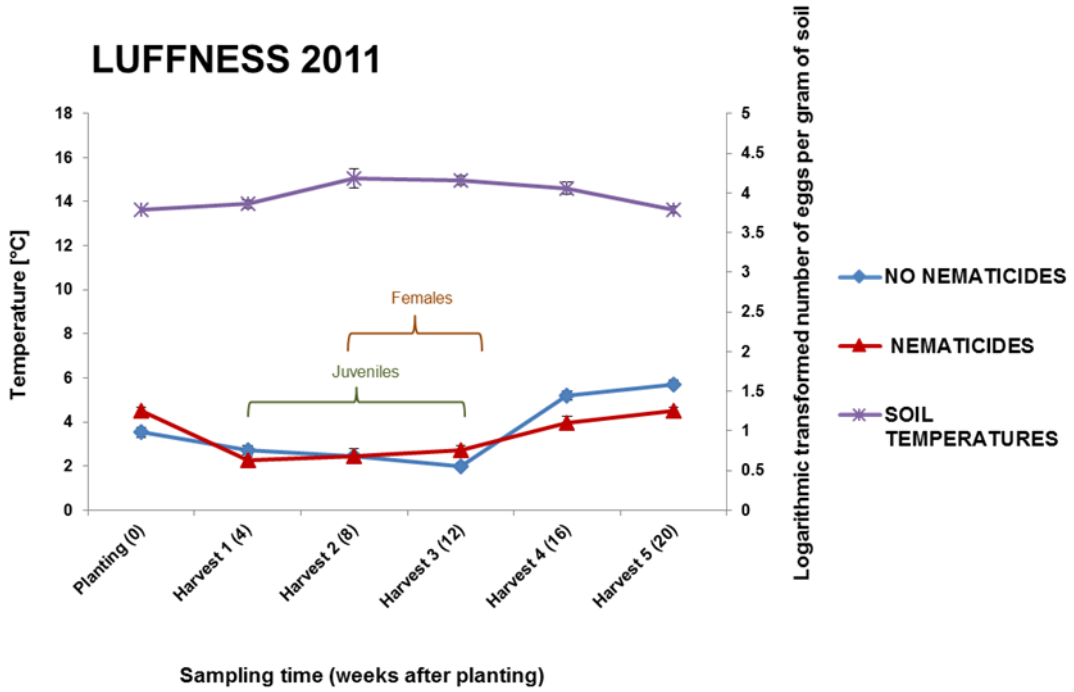

Figure 3.34 Comparison of the different developmental stages of PCN determined by qPCR and observation inside acid fuchsin stained roots of cultivars Cara and Desirée at Luffness in 2011 field trial. Primary y axis is the mean temperatures between the harvests. Secondary y axis is the logarithmic transformed number of eggs/g soil determined by qPCR over the growing season from untreated and nematicide treated plots over the 22 week growing period. Juveniles stages (J2, J3, J4) were combined and are presented as "Juveniles". Root samples were examined at harvests 1, 2, 3, 4, (weeks 4, 8, 12, and 16) at weeks 20 and 22 the roots had rotted. The bars are standard errors of the means of four cultivars used for this study and mean of temperatures per 4 week period between each harvest.

3.4.6. Yield

3.4.6.1. Luffness 2011

The biggest component of yield with respect to weight consisted of tubers sized 45–65 mm for all four cultivars (Cara, Desirée, Estima and Maris Piper) and the number of tubers below 65 mm for all tested cultivars. Figure 3.35 and Figure 3.36 illustrate the weight of tubers and number of tubers in each size group for the 4 cultivars with and without nematicide treatment. Figure 3.37 and Figure 3.38 present the total combined yield and total number of tubers for the 4 cultivars with and without nematicides. Further statistical tests (ANOVA) revealed significant differences between cultivars in total yield ($P<0.001$) and in total number of tubers ($P=0.011$). No significant differences between nematicide and no nematicide treatments were observed in total yield ($P=0.731$) or in number of tubers ($P=0.317$). However, the nematicide cultivar interaction showed that the cultivars responded in a significantly different way to the nematicide treatments ($P<0.001$). Cara had significantly higher yields in the nematicide treated plots compared to those that were untreated and surprisingly, Maris Piper and Estima yielded higher in untreated plots than with nematicide. There were significant differences between the nematicide treatments and cultivars.

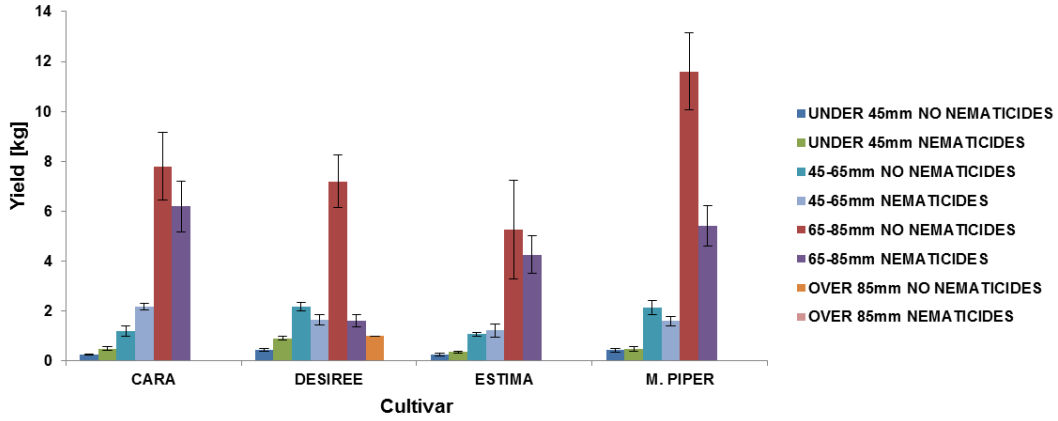

Figure 3.35 Yield in weight of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 20 weeks at Luffness in 2011.

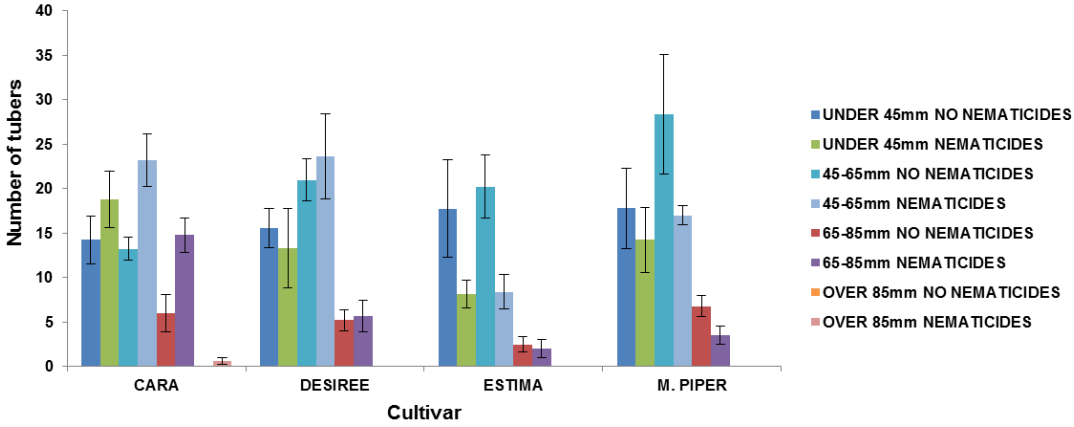

Figure 3.36 Number of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 20 weeks at Luffness in 2011.

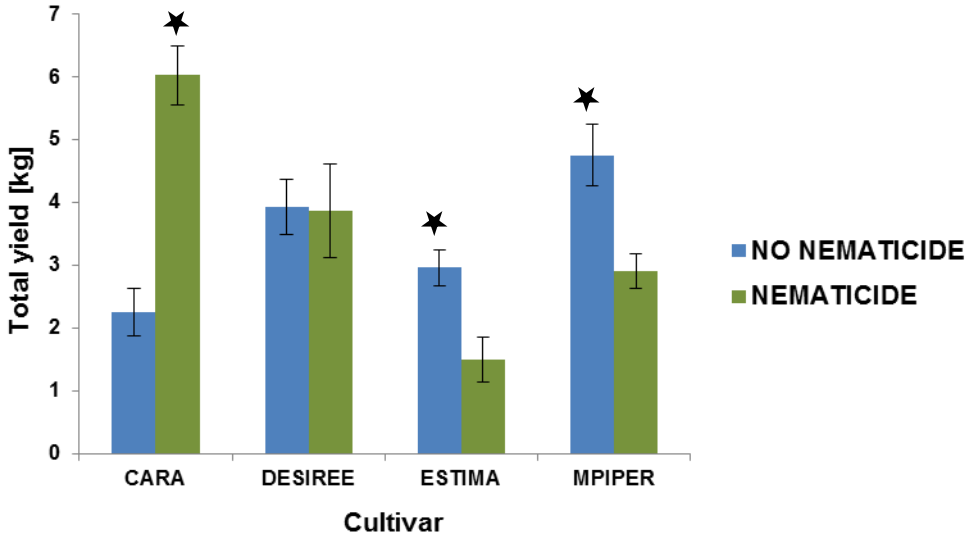

Figure 3.37 Total yield of tubers for the cultivars Cara, Desirée, Estima and Maris Piper at Luffness in 2011. The bars are standard errors of the means for each cultivar.

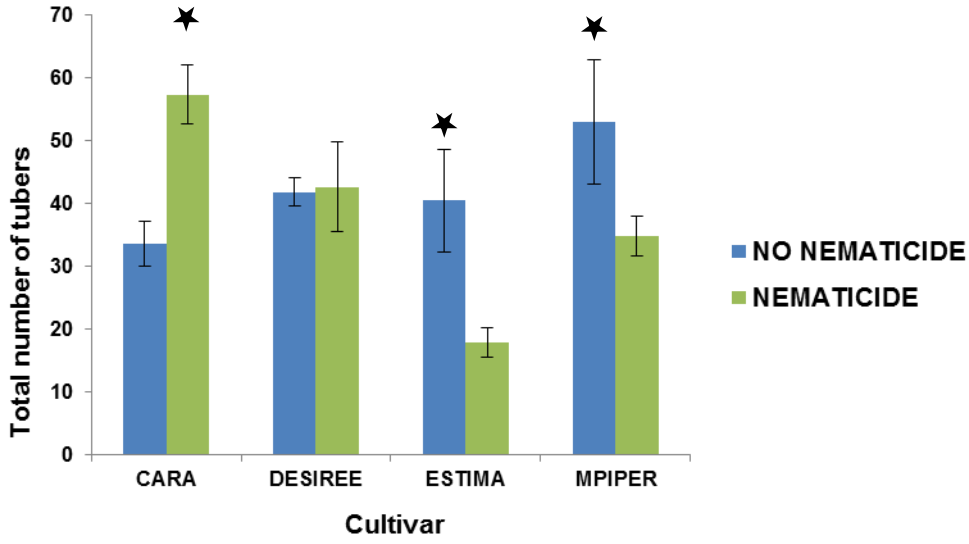

Figure 3.38 Total number of tubers for the cultivars Cara, Desirée, Estima and Maris Piper at Luffness in 2011. The bars are standard errors of the means for each cultivar.

3.4.6.2. Luffness 2012

The biggest component of yield with respect to weight consisted of tubers sized 45–65 mm for three cultivars (Desirée, Vales Everest and Maris Piper), for cv Cara yield in each group size had the same values. The highest number of tubers was below 65 mm for all four tested cultivars. Figure 3.39 and Figure 3.40 demonstrate the weight of tubers and number of tubers in each size group for the 4 cultivars with and without nematicide treatment. Figure 3.41 and Figure 3.42 present the total combined yield and total number of tubers for the 4 cultivars with and without nematicides. Statistical tests (ANOVA) indicated that there were no significant differences between nematicides and no nematicides treatments ($P=0.219$) or between cultivars (Figure 3.41) and (Figure 3.42) ($P=0.171$). There was also no significant difference in response of cultivars to nematicides ($P=0.314$) in the total yield. However, there was a significant difference between cultivars in terms of number of tubers ($P<0.001$).

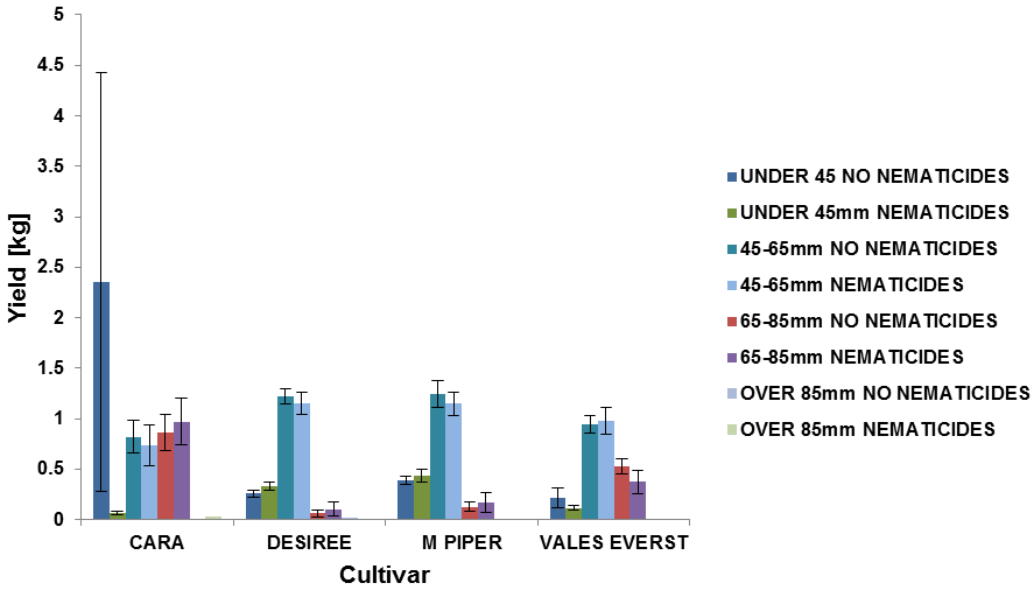

Figure 3.39 Yield in weight of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 22 weeks at Luffness site in 2012. The bars are standard errors of the means for each cultivar.

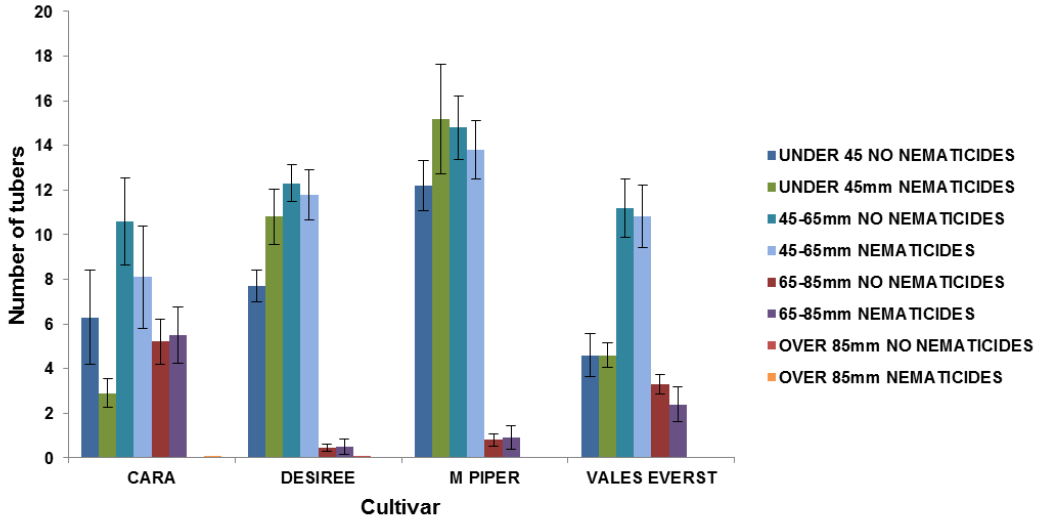

Figure 3.40 Yield in number of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots

after 22 weeks at Luffness site in 2012. The bars are standard errors of the means for each cultivar.

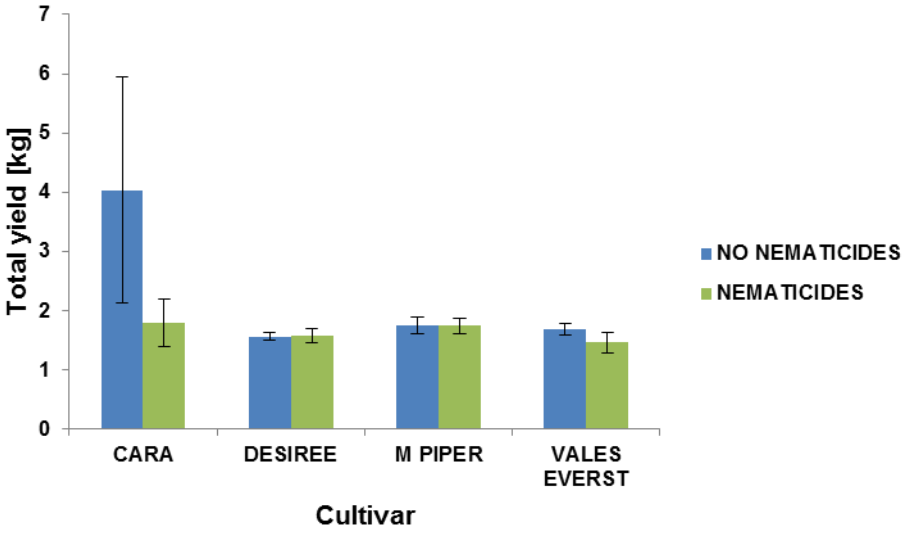

Figure 3.41 Total yield of tubers for the cultivars Cara, Desirée, Estima and Maris Piper at Luffness site in 2012. The bars are standard errors of the means for each cultivar.

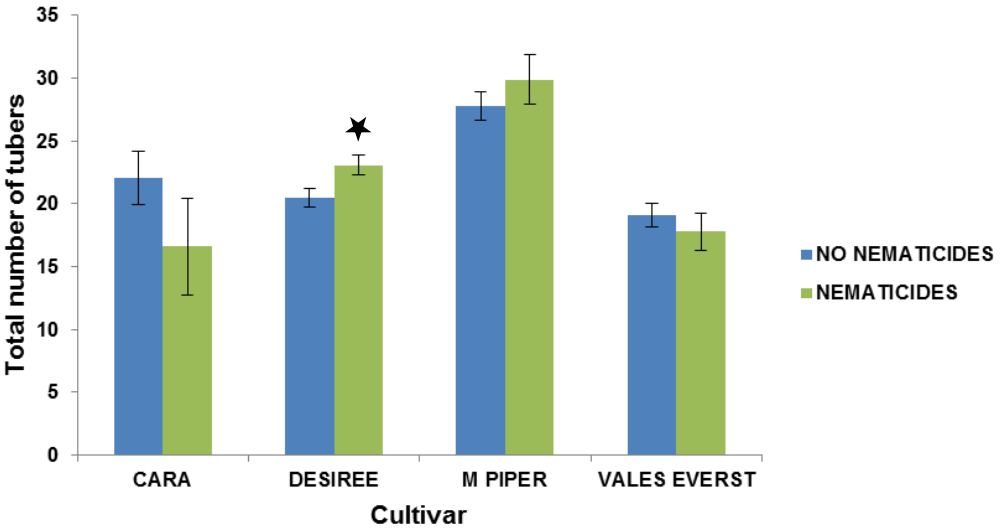

Figure 3.42 Total number of tubers for the cultivars Cara, Desirée, Estima and Maris Piper at Luffness site 2012. The bars are standard errors of the means for each cultivar

3.4.6.3. Harper Adams 2011

As found at Luffness, at Harper Adams the highest yield was recorded in the 45–65 mm size group and with respect to number of tubers below 65 mm for all four tested cultivars; however there were no tubers >85mm (Figure 3.43 and Figure 3.44); the total yield and total number of tubers for the four cultivars with and without nematicide treatment respectively are shown in Figure 3.45 and Figure 3.46.

Statistical analysis performed on the yield results showed no significant differences either in number of tubers (P= 0.237) or total yield (P=0.835) between the cultivars.

All the cultivars had significantly lower yields for the untreated plots compared to the treated (P=0.013), nevertheless the number of tubers did not differ significantly between treated and untreated plots (P=0.709). There was also no significant nematicide × cultivar interaction in yield (P=0.853) and number of tubers (P=0.649), indicating that the cultivars responded similarly in the nematicide and no nematicide environment.

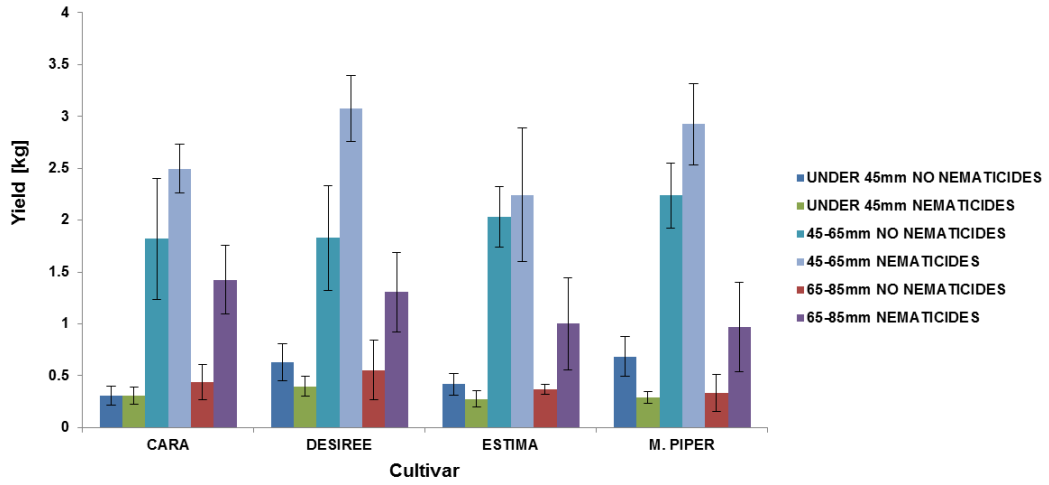

Figure 3.43 Yield in weight of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 22 weeks at Harper Adams in 2011. The bars are standard errors of the means for each cultivar.

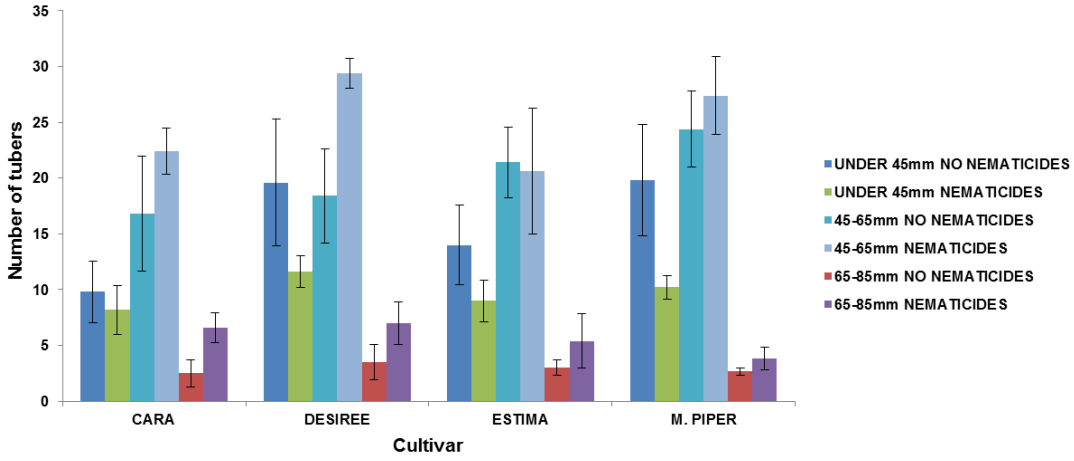

Figure 3.44 Yield in number of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 22 weeks at Harper Adams in 2011. The bars are standard errors of the means for each cultivar..

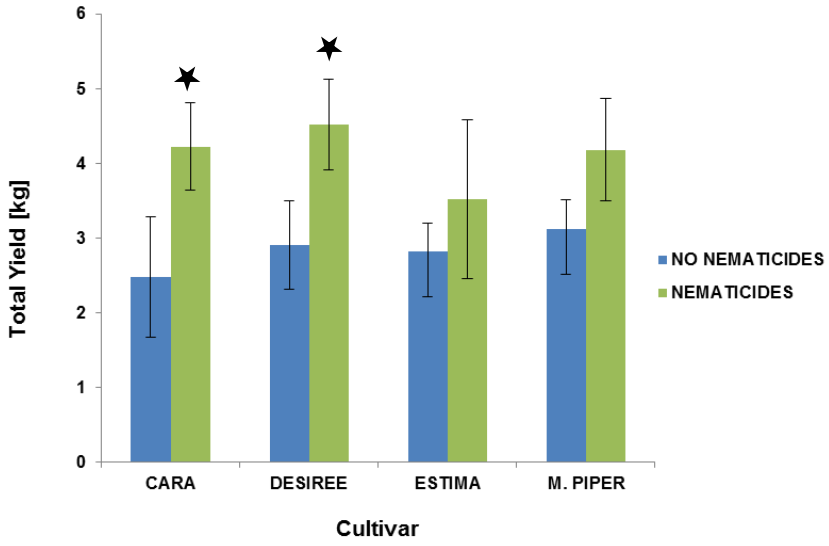

Figure 3.45 Yield Harper Adams in 2011 from untreated and nematicide treated plots after 22 weeks. The bars are standard errors of the means for the each cultivar.

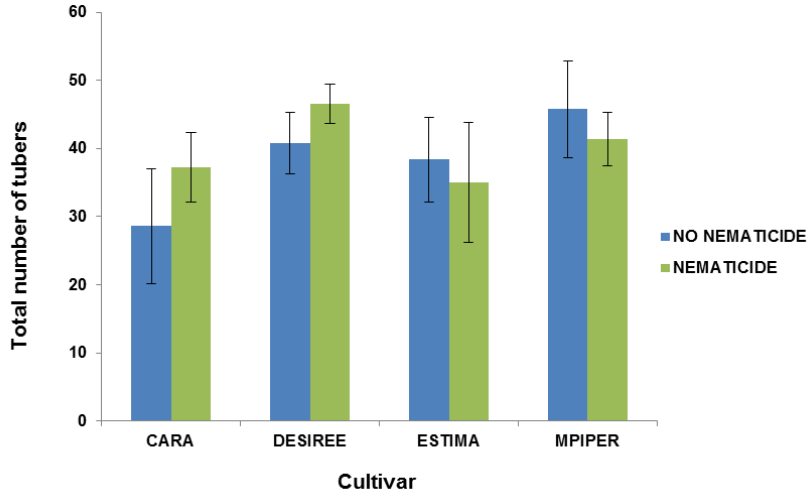

Figure 3.46 Total number of tubers at Harper Adams in 2011 from untreated and nematicide treated plots after 22 weeks. The bars are standard errors of the means for the each cultivar.

3.4.6.4. Harper Adams 2012

Similar to Luffness, the biggest component of yield with respect to weight consisted of tubers sized 45–65 mm for all four cultivars (Cara, Desirée, Vales Everest and Maris Piper). The highest number of tubers recorded was below 65 mm for all tested cultivars (Figure 3.47 and Figure 3.48). The cultivars differed significantly (P=0.021) in total yield, but not between number of tubers (P=0.144). Nematicide application did not increase either the yield (P=0.205) or the numbers of tubers (P=0.951) as expected. There also was no significant nematicide × cultivar interaction regarding their response to nematicides in yield (P=0.293) or number of tubers (P=0.337) (Figure 3.49 and Figure 3.50).

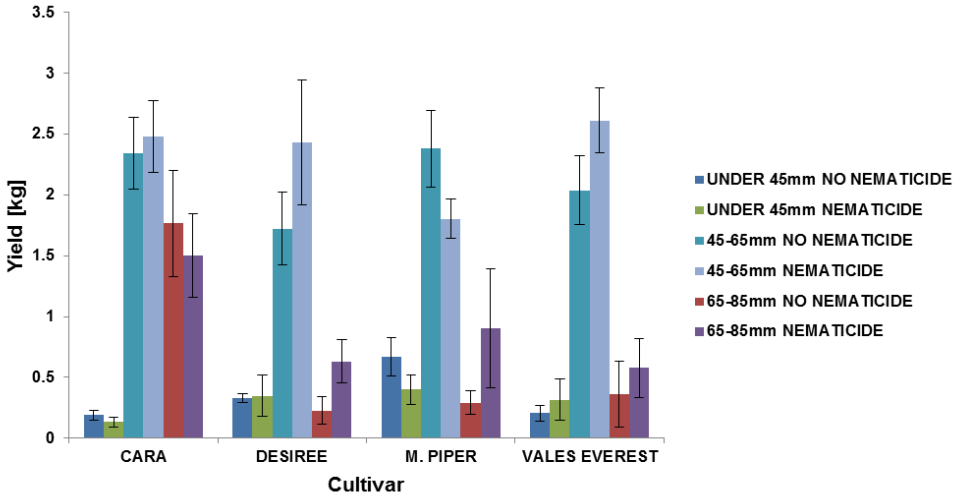

Figure 3.47 Yield of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 26 weeks at Harper Adams in 2012. The bars are standard errors of the means for the each cultivar

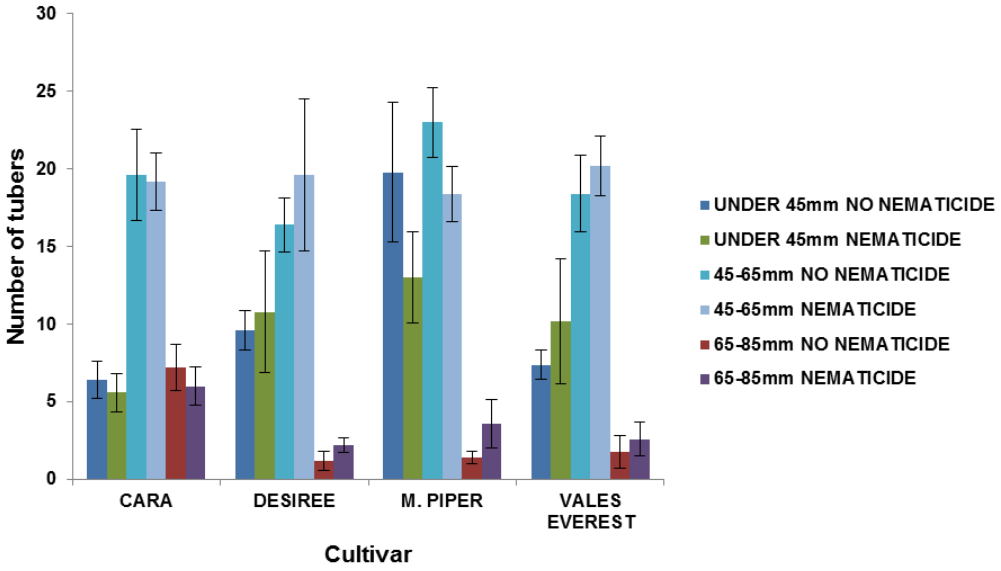

Figure 3.48 Yield of tubers from cvs Cara, Desirée, Estima and Maris Piper in size groups (<45, 45-65, 65-85 and >85mm) in untreated and nematicide treated plots after 26 weeks at Harper Adams in 2012. The bars are standard errors of the means for the each cultivar

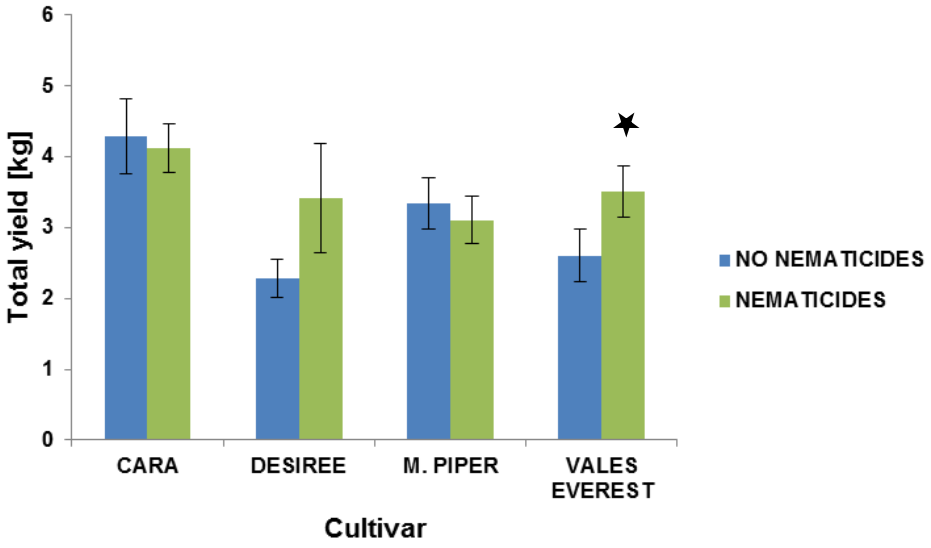

Figure 3.49 Total yield in weight of tubers at Harper Adams in 2012 from untreated and nematicide treated plots after 26 weeks. The bars are standard errors of the means for the each cultivar

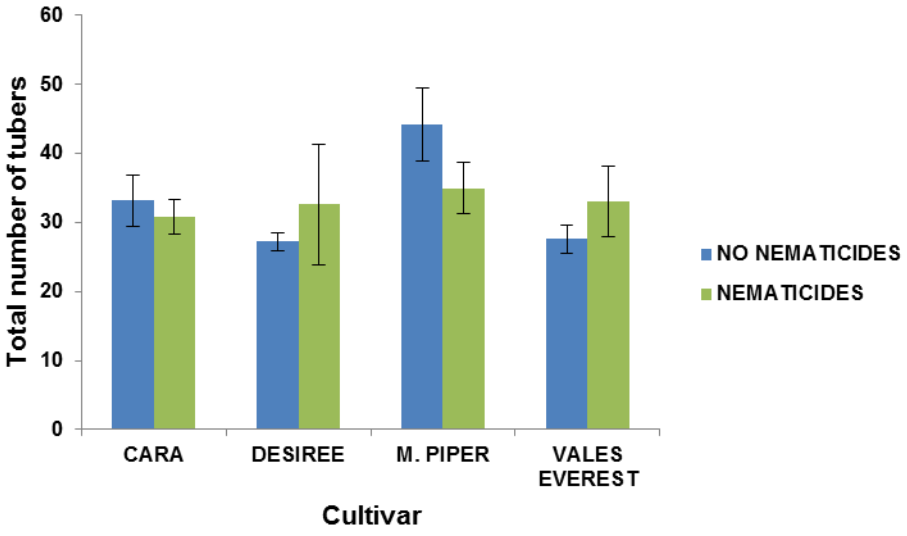

Figure 3.50 Total number of tubers at Harper Adams in 2012 from untreated and nematicide treated plots after 26 weeks. The bars are standard errors of the means for the each cultivar

3.4.6.5. Relationship between initial population and yield

Due to very low infestation in the plots from 2012 it was almost impossible to investigate the relationship between initial population and yield. Below are results from experimental plots in 2011. For the purpose of ANOVA the infestations were categorized to be light (<5 eggs/g soil), moderate (5–20) or heavy (>20 eggs/g soil). The Harper Adams site was only lightly to moderately infested with *G. pallida* and the Luffness site from moderately to heavily infested. The ANOVA on the yield with the cultivar, nematicide and initial infestation as factors showed significant influence of the initial population on yield (P=0.035) and a significant interaction between cultivar and initial infestation (P=0.027) (Table 3-10). Figure 3.51 presents the relationship between total yield and initial population (Pi). The yield of cv Cara was not affected by the Pi in the untreated plots. In the treated plots there was a slight trend of increasing yield. The cv Desirée showed a clear reduction of yield when the Pis were increasing in the untreated plots. With nematicide treatment there was an increase in the yield, however the trend of decreasing yield with increasing population remained. For Pis greater than 10 eggs/g soil cv Estima had lower yield in plots with or without nematicide. No trend in cv Maris Piper was found in yield in terms of initial population.

Experimental plots at Luffness site had a good range in the population densities (<1 to > 45 eggs/g soil). The ANOVA of the yield with the cultivar, nematicide and initial infestation as factors showed no significant influence of initial population on yield (P=0.554) and any interaction between cultivar and the

initial infestations was at the limits of significance (P=0.053) (Table 3-11). Only cv Estima resulted in a slight decrease in yield in terms of initial population. The rest of the cultivars did not show any clear trend of reduction of yield with increasing density of initial population.

Table 3-10 Analysis of variance (ANOVA) of the yield from the Harper Adams site 2011 with the cultivar, nematicide and initial infestation as factors with *P*<0.05.

Source of variation	Degrees of freedom	Sums of squares	Means squared	Variance	F probability
Cultivar	3	2.06	0.687	0.39	0.763
Infestation	1	8.779	8.779	4.95	**0.035**
Nematicides	1	11.954	11.954	6.74	**0.015**
Cultivar x Infestation	2	14.691	7.346	4.14	**0.027**
Cultivar x Nematicides	3	7.29	2.43	1.37	0.274
Residual	26	46.079	1.772		
Total	36	90.853	2.524		

Table 3-11 Analysis of variance (ANOVA) of the yield from the Luffness site 2011 with the cultivar, nematicide and initial infestation as factors with *P*<0.05.

Source of variation	Degrees of freedom	Sums of squares	Means squared	Variance	F probability
Cultivar	3	29.781	9.927	7.06	**0.001**
Infestation	1	0.507	0.507	0.36	0.554
Nematicides	1	1.334	1.334	0.95	0.34
Cultivar x Infestation	3	12.427	4.142	2.95	0.053
Cultivar x Nematicides	2	14.595	7.297	5.19	**0.013**
Residual	24	33.722	1.405		
Total	34	92.366	2.717		

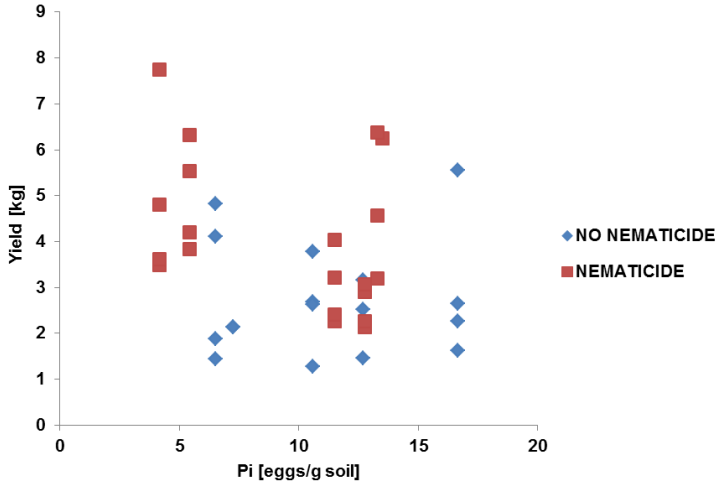

Figure 3.51 Relationship between total weight of yield and initial density at Harper Adams in 2011 on four different genotypes of potato (cvs Cara, Desirée, Estima and Maris Piper). Yield (kg) is plotted against the population density at planting (Pi) in untreated (blue) and nematicide treated (red) experimental plots.

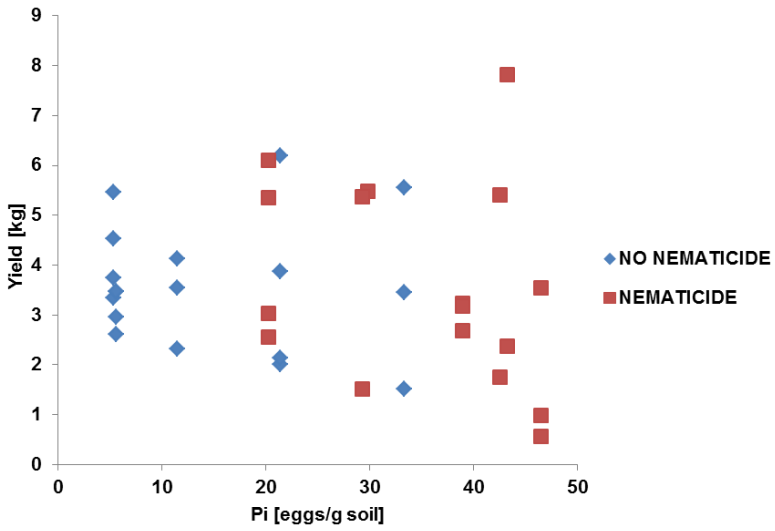

Figure 3.52 Relationship between total weight of yield and initial density at Luffness in 2011 on four different genotypes of potato (cvs Cara, Desirée , Estima and Maris Piper). Yield (kg) is plotted against the population density at planting (Pi) in untreated (blue) and nematicide treated (red) experimental plots.

3.5. Discussion

The main goals of the experiments described in this chapter were to examine the relationship between the potato cyst nematode mulitplication at different initial population densities, with different species composition and in different agroecological conditions.

One of the factors affecting PCN population reproduction is competition between two species present for food or feeding sites. The first experiment aimed to investigate the interspecific competition on different host plants and was performed with susceptible cultivar Desirée, fully resistant to *G. rostochiensis* but susceptible to *G. pallida,* cv Maris Piper (*H1*) and the partially resistant to both species cv Vales Everest with resistance derived from *S. tuberosum* spp. *andigena* (CPC 2802). The pot experiment was performed by using suspensions of eggs of either PCN species or mixtures of the two species which allowed different combinations and densities of inocula to be created. As mentioned in the introduction, competition between the species is density dependent. The final number of eggs and the multiplication ratio (Pf/Pi) were used to determine the interaction between species on the hosts with different resistances. Unexpectedly there was a small amount of cross contamination seen with qPCR in the inoculum containing purely eggs of *G. rostochiensis*; which possibly happened during multiplying cysts for the JHI collection. In the author's opinion it was too small to affect the development of population A.

The results showed a significant reduction in the multiplication rate of both species when inoculated as mixtures on cvs Desirée and Vales Everest. A decrease in *G. rostochiensis* multiplication rate was found when *G. pallida* was

present in higher or equal initial densities on cvs Desirée and Vales Everest. The opposite effect was observed on cv Vales Everest when the main species in the inoculum was *G. rostochiensis* suggesting that resistance combined with high density of *G. rostochiensis* population significantly reduced reproduction rate of *G. pallida*. One explanation for this could be related with differences in the responses and development the two PCN species. Differences in hatching and developmental rates were described in Chapter 2. However, even though *G. rostochiensis* hatched and completed the life cycle more quickly than *G. pallida,* the latter species *G. pallida* had a significantly higher overall amount of hatching and surpassed *G. rostochiensis* in both hatching and multiplication confirming the hypothesis stated by den Nijs 1992, who claimed that *G. pallida,* by their higher efficiency, probably reduced feeding sites in the roots for *G. rostochiensis* development. This effect was particularly noticeable with the susceptible Desirée when the initial density of *G. pallida* was much higher than *G. rostochiensis*. Moreover, both species were equally affected and their multiplication rates were reduced when the food availability was reduced (resistant cultivar) and inoculum consisted of exactly the same proportions of both species.

The field trial sites were located in two different agroecosystems and provided an opportunity to relate the results from the life cycle experiments performed in controlled conditions to two geographically separated field sites in the UK. The fields were both infested by *G. pallida;* however, as mentioned in the results section, a small infestation of *Globodera rostochiensis* was found in the experimental plots from 2012 at Luffness.

The results from 2011 show that *Globodera* egg densities changed within the growing season and at the different locations. In 2011 all of the cultivars used were susceptible to *G. pallida* and no significant difference in the final multiplication rate (Pf/Pi) between cultivars was seen at either site. However, in the Harper Adams trial, cvs Cara and Estima showed declines in the egg/g levels in the untreated field after 16 weeks of planting in contrast to Desirée and M. Piper which corresponded with the observation of juveniles in the roots of Cara. The reason for this is not clear but it may related to the physiology of cvs Cara and Estima. Cara is the late cultivar and is known to have a large root system and to be tolerant to infestation by PCN. Another question to be asked is why this distinction was not seen at Luffness.

Nematode population dynamics are density dependent and are influenced by many environmental factors, i.e. soil temperature, rainfall or soil type as well as host growth and other agronomic factors. One of the methods for controlling PCN populations is the treatment with nematicides which can be either nematostatic and/or nematicidal. They can affect nematodes during different developmental stages: first in the dormant larval stage when eggs can be killed in the cyst; second during hatching of the juveniles by inhibition and also by disruption of movement during localisation of the roots as well as killing juveniles while moving in the soil to the roots from cyst (Haydock *et al*., 2013). Nematicides can also have an effect on the juveniles that have managed to invade the roots (Haydock *et al*., 2013). Woods *et al.* (1999) discovered that fosthiazate temporarily inhibited hatching of *G. pallida* in an *in vitro* test and suppressed hatching in soil. Data from other field experiments (Evans, 1982;

Woods *et al.,* 1999) also suggest that carbamate nematicides were not able to permanently stop hatching in field conditions, but that they delayed the process significantly. The results from the trials at Luffness and Harper Adams showed a significant response in the egg numbers with and without nematicide treatment and with the number of weeks of growing potatoes. Interestingly, there was no observed effect of the nematicides on juvenile root invasion. This agrees with observations by Minnis *et al*. (2004), who showed that oxamyl did not affect root invasion. In contrast to the observations with the juvenile stages, the numbers of females differed with and without nematicide treatments at both sites.

Another factor influencing population dynamics in the field is the level of initial infestation. There are reports that the initial population Pi is negatively correlated with the rate of *G. rostochiensis* reproduction (LaMondia and Brodie, 1986). The observed multiplication rates differed significantly between sites and years. The main source of difference at the Luffness site was with and without nematicide treatment ($P<0.001$). However, as mentioned previously, the density of the initial population in untreated plots was between 5–20 eggs/g soil while the plots for nematicide treatment had >20 eggs/g 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. At Harper Adams the Pf/Pi did not differ between treated and untreated plots or between cultivars.

As mentioned above, at Luffness the Vydate (oxamyl) treatment reduced the population significantly contrasting with results obtained by Halford *et al*. (1999) who showed no significant difference between fields treated and untreated with

oxamyl. In contrast, at Harper Adams no significant difference between population multiplication with fosthiazate and untreated plots was observed. In 2012 the multiplication rate was extremely high at Harper Adams on the untreated fields with an untransformed Pf/Pi ratio increased 550X and in treated fields 250x with the *Pi* <1 egg/gm of soil confirming results obtained by Trudgill *et al*. (2014). In 2012, Vydate was used as a control method at both sites, and there was no difference in multiplication rates between the nematicide and no nematicide treated plots. In both treatments the Pf/Pi rate was significantly higher at Harper Adams than at Luffness. Nevertheless at Harper Adams cv Vales Everest slowed down multiplication of PCN in the field and achieved the lower Pf/Pi rate than other cultivars, although this effect was not observed at Luffness. There are several possible explanations for this result. One of them might be related with the virulence difference between two populations of *G. pallida* (Phillips *et al.,* 1991; Trudgill *et al.,* 2014) but also the generally poor weather in 2012. Low initial Pi was also not helpful for revealing differences between the cultivars.

Changes in the population density can also affect the yield (Seinhorst 1980; Seinhorst 1965) and there is a correlation between yield loss and the initial population level (Elston *et al.,* 1991; Trudgill, 1986; Trudgill *et al.,* 2014). Root invasion by PCN results in damage to the root system and causes disruption in root growth and a decrease in the ability to take up nutrients from soil, resulting in lower yields. In most cases, a higher invasion rate results in lower yields (Trudgill, 1986; Phillips *et al*., 1998; Minnis *et al.,* 2004). The first models describing the relationship between initial population level and yield suggested

a linear response of yield loss in terms of increasing population of PCN in the field (Oostenbrink, 1968; Brown, 1983). With the field trials in 2011 reported in this chapter, the ANOVA showed for the same Pi, total yield did not differ significantly, however there was a general response of the cultivars to the initial population level and a negative trend was observed in Desirée and Estima in Harper Adams and Maris Piper in Luffness; with increasing initial PCN population the yield losses were higher similar to results published by Trudgill *et al.,* (2014). In 2011 there were also differences in yield at the Luffness site. Cara had significantly higher yields in the nematicide treated plots compared to those that were untreated and, surprisingly, Maris Piper and Estima yielded higher in untreated plots than with nematicide In contrast, at Harper Adams, none of the factors (cultivar or nematicide) significantly influenced the yield. In 2012 yield did not differ between cultivars or nematicide treatment and was higher than in 2011, and the initial populations of PCN were low in 2012. The reason for these results might also be related to non-PCN related factors such as weather (soil temperature, rainfall).

The soil temperature profiles differed between the two sites during the growing season. At Luffness the temperatures varied from 10 to 18.5°C with a mean of 14.2˚C, whereas at Harper Adams they ranged from 9.5 to 19.4°C with a mean of 14.9˚C and were also significantly higher at Harper Adams than in Luffness at the latter part of the growing season The number of juveniles found at the end of growing season might suggest the diapause stage was broken due to higher temperatures and that a second generation began developing. Kaczmarek *et al.* 2014 predicted hatching at Harper Adams in England and Whitewater in

Scotland with different soil temperature profiles. They reported that soil temperature affects hatching, and modelled that at Harper Adams, which was a warmer site, hatching was predicted to be faster for both species of PCN than at cooler Whitewater.

Tiilikkala (1987) studied the development of the *G. rostochiensis* populations in the field in Finland during 1981–1984. He concluded that PCN is well adapted to the low ground temperatures in Finland and has the ability to multiply over the growing season in the regions up to the polar circle. He also reported differences between populations from different locations. Greco (1988) also compared the length of life cycle of PCN in two different regions: colder Catania and warmer Bari and reported that in Italy *G. rostochiensis* was able to produce a second generation on slow maturating potatoes in Bari, which greatly increased the final population. However that did not happen in Catania, where potatoes matured before the second generation started. Greco and Moreno (1992) also noted that if potato crops were planted continuously, *G. rostochiensis* skipped the diapause stage and developed a second generation during one season in Chile.

The results reported in Chapter 2 clearly show that warmer soil temperatures not only increased the rate of hatching for both species but also increased the overall amount of hatching. This, it can be assumed, will lead to increased population levels on susceptible hosts and more damage to the crop. 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 rates and have

greater challenges in controlling population levels. However, due to the low infestation level of the experimental plots in the field studies in 2012 and poor weather it was not possible to reliably analyse and estimate the probability values for changes within the growing season, thus the interactions between temperature and population dynamics that year as well as density dependence were not distinguished.

4. GENOTYPING FIELD POPULATIONS

4.1. Introduction

This chapter concerns the molecular characterisation of *G. pallida* which was the dominant species in the field experiments presented in Chapter 3. Chapter 2 and 3 describe differences between the two species in their responses to temperatures and their performance on different cultivars. These differences may be related to the pathotype of PCN that was tested. For example Foot (1978) described significant differences between pathotypes of *G. pallida* Pa2 and Pa3, and reported that pathotype Pa2 was better adapted to lower temperatures than Pa3. Franco (1979) also presented results suggesting that some pathotypes differed in their responses to temperature. He tested Peruvian (Otuzco) and British populations of *G. pallida* and showed that the Peruvian population had higher hatching rate than the British at all tested temperatures. Another important observation from his research was that the number of eggs varied in different temperatures; the highest number was obtained at 14 and 18°C for the British and the Otuzco populations respectively. In addition the optimal temperatures for root invasion were at 14 and 10°C for the British and Peruvian populations respectively. His results suggest that the population from Otuzco had a broader temperature optimum than the British population. Turner *et al.* (2009) also described different hatching behaviour between pathotypes and reported significantly lower hatch of Pa1 pathotype than populations classified as a pathotype Pa2, Pa3 and Pa2/3. In Chapter 1 pathotyping

methods are described and the requirement for alternative approaches that are faster in distinguishing pathotypes.

Various studies have been conducted on the genetic variability of *G. pallida* populations (Picard *et al*., 2007; Plantard *et al*., 2008) and this molecular characterisation has helped to reveall the diversity, taxonomy and phylogeny of PCN and is now being used to design molecular methods of pathotyping.

European and South American populations of PCN were studied using various biochemical techniques such as isozymes (Fleming and Marks 1983; Fox and Atkinson, 1984), two dimensional electrophoresis (Bakker *et al*., 1992), restriction fragment length polymorphism (RFLP) (Schnick *et al.,*1990; Phillips *et al*., 1992) and Random Amplified Polymorphic DNA (RAPD) (Williams *et al*., 1990; Blok *et al.,*1997). More recently, genetic variation has been investigated using various molecular markers including the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) (Blok *et al*., 1998; Subbotin *et al*., 2000; Pylypenko *et al*., 2005), microsatellites (Grenier *et al*., 2001) and the mitochondrial cytochrome B (cytB) *(*Picard *et al*., 2004; Picard and Plantard, 2006; Picard *et al.,* 2007; Plantard *et al.*, 2008; Pylypenko *et al*., 2008) or in combination (Madani *et al*., 2010; Hoolahan *et al*., 2012).

This chapter examines variation within and between the populations found during the field experiments (Chapter 3) and from samples obtained from other land in which potatoes had been cropped in the vicinity. The methods used in this study for population identification were based on a noncoding region of *G. pallida* scmtDNA IV previously described by Armstrong *et al.*, (2007) and CytB (Picard *et al.,* 2007; Plantard *et al.*, 2008). Phylogenetic analyses of CytB in

combination with other markers have previously been shown to be able to distinguish most of South American and European populations of *G. pallida* (Picard *et al*., 2007; Plantard *et al*., 2008; Madani *et al*., 2010; Hoolahan *et al*., 2012). According to Hoolahan *et al*., (2012) use of one individual marker may be inadequate for differentiating populations of *G. pallida* and application of analysis with non-coding mtDNA marker could deliver more information about orgin and distribution of *G. pallida*, and also provide a new diagnostic tool.

The PCR-RFLP of the mitochondrial non-coding region was developed into a diagnostic method by Grujić (2010). Primers were designed to amplify part of the non-coding region of scmtIV (s222) and after digestion with the restriction enzyme TaqI three types of restriction band patterns were identified which are found in UK populations of *G. pallida* (Pa1, Pa2/3 (type E Lindley) Pa2/3 (type Luffness)) previously characterised by Phillips *et al.* (1992), Blok *et al.,* (1998) and Phillips and Trudgill (1998).

4.2. Material and methods

4.2.1. Nematode populations

Ten populations of *G. pallida* from the United Kingdom were examined in this study: four were sampled over the two years from the experimental plots in Luffness, Scotland and Harper Adams, England (Chapter 3), three were sampled from the three different fields in East Lothian region, Scotland and three were provided by Jeremy Cartwright (Harper Adams University), two from Shropshire and one from Herefordshire, England (Table 8-2).

4.2.3. DNA extraction

Ten single cysts per population were picked using a microscope and genomic DNA was extracted from each with MicroLysis Plus (Microzone, Cambridge, UK). A single cyst was placed in a 1.5 ml Eppendorf tube with 20 µl of MicroLysis Plus, and cysts were crushed in the buffer using a plastic homogenizer for 5 min. Samples were then centrifuged for 1 min at 8000 g, the supernatant transferred to a new tube and then processed according to manufacturer's recommendations (Microzone Limited, UK).

4.2.4. Polymerase chain reaction

Extracted DNA was initially tested with species diagnostic primers (Table 4-1) to distinguish the PCN species. The 10 µl PCR reactions contained: 0.04 µl (0.2 units) Taq polymerase (Promega, Southampton, UK), 2.0 µl 5x buffer, 0.4 µl of

the specific primers (PITp3 and PITSr4) for each species of PCN (at 10 µM/µl), 0.4 µl of UNI primer (at 10 µM/µl), 0.6 µl $MgCl_2$, 4.16 µl HPLC H_2O (Sigma), 1 µl of 0.2 mM dNTPs and 1 µl DNA from cysts extracted previously or H_2O for the negative control.

Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Warrington, UK), with the following cycling conditions; denaturation at 94°C for 5 min followed by 40 cycles of 94°C for 30 s, 55°C for 15 s and 72°C for 30 s and a final elongation at 72°C for 5 min. The amplified products were separated by electrophoresis in a 1.5% agarose gel buffered with 1% Tris-Borate-EDTA (TBE) with Sybr-safe dye, and visualised with UV illumination.

4.2.5. PCR RFLP

Positive samples of *G. pallida* were then used for amplification of the non-coding region of scmtDNA IV (s222) with primers F3mtDNA 222 and scmt 4-8 (Table 4.2) using PureTaq Ready-To-Go PCR Beads (GE Healthcare UK Ltd, Little Chalfont, UK) in 25 µl PCR reactions containing 21 µl HPLC water, 1 µl of each primer and 2 µl of template DNA prepared as described in 4.2.3. The PCR conditions were: 94°C for 2 min followed by 40 cycles at 94°C for 15 s, 55°C for 15 s, 72°C for 1 min with an extension at 72°C for 5 min followed by a hold at 20°C.

5 µl of the PCR products were loaded in a 1.5% TBE agarose gel to check the size of the PCR products. The remaining 20 µl from reactions was purified using MinEluteQuick PCR purification kit (Qiagen, Crawley, West Sussex, UK) and

digested with 2 µl (20 units) restriction enzyme *TaqI* (Promega, Southampton, UK), 3 µl 10x E buffer provided with restriction enzyme and 5 µl of HPLC water, for 3 hours at 65°C. Loading dye was added to the digestion mix which was loaded on a 2% TBE agarose gel to obtain an RFLP image. The digested products were separated by electrophoresis and visualised with UV illumination. As controls, plasmid DNA from clones of the three amplification types previously obtained from different populations from the JHI PCN collection were also amplified and digested as described above (Grujić, 2010).

4.2.6. Cytochrome B sequencing

Two specific primers INRAcytbR and INRAcytbL(Table 4-1) were used to amplify most of the CytB gene (Picard *et al.,* 2007). The CytB amplification was carried out using PureTaq Ready-To-Go PCR Beads (GE Healthcare UK Ltd, Little Chalfont, UK) in a 25 µl PCR reaction containing 21 µl HPLC water, 1 µl of each primer and 2 µl of template DNA. Amplification conditions were: 94°C for 2 min followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s with the extension at 72°C for 5 min and a hold at 20°C. 25 µl of the PCR product was loaded in 2% agarose gel in TBE buffer.

The CytB PCR products were extracted from the agarose gel using a sterile scalpel and purified with a Qiagen MinElute Gel Extraction Kit (Qiagen, Crawley, West Sussex, UK) according to manufacturer's protocol. The purified DNA concentration was measured using a NanoDrop spectrophotometer (ThermoScientific, Wilmington, USA), appropriately diluted, and sequenced in

the JHI sequencing facility in both directions using primers INRAcytbR and INRAcytbL.

Table 4-1 List of the primers used in this study, their sequences, use and sources.

Primer	Primer sequence	Use of primers	Source
PITSr3	5'-AGCGCAGACATGCCGCAA-3'	Distinguishing PCN species	(Bulman and Marshall, 1997),
PITSp4	5'-ACAACAGCAATCGTCGAG-3'	Distinguishing PCN species	Bulman and Marshall, 1997
UNI	5'-CGTAACAAGGTAGCTGTAG-3'	Distinguishing PCN species	(Ferris *et al.,* 1993)
F3mtDNA 222	5-ATTAGACCGATAAGTTTACACCTTG-3'	S222 noncoding region	(Grujić, 2010)
scmt 4-8	5'-GACTAGGTCCATCAATCTGAACC-3'	S222 noncoding region	(Grujić 2010)
INRAcytbL	5'-GGGTGTGGCCTTGTTATTTC-3'	CytB gene amplification	(Picard *et al*. 2007)
INRAcytbR	5'-ACCAGCTAAAACCCCATCCT-3'	CytB gene amplification	(Picard *et al*. 2007)

4.2.7. Bioinformatic analysis

The CytB sequences obtained were edited and consensus sequences of forward and reverse sequences produced using Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, USA) and Jalview (Clamp *et al*., 2004). Sequences

were aligned with those obtained from *G. pallida* populations in the JHI PCN collection including UK, European and S. America populations or from the NCBI database. The phylogenetic analysis was performed by constructing a maximum likelihood tree using the HKY model using TOPALi (Milne *et al.,* 2004). Bootstrap analyses were based on 1000 iterations. The phylogenetic tree was rooted with *G. rostochiensis* (JHI sequence collection) and *G. mexicana* (Plantard *et al.*, 2008) CytB sequences as outgroups and edited in FigTree v1.4.0 (Rambaut, 2009).

4.3. Results

4.3.1. PCR RFLP

The comparison between field populations and the three plasmid clone types (Lindley, Luffness and Pa1) is shown in Figure 4.1. Cysts extracted from the fields located in the East Lothian region showed three groups of digestion patterns. The field used for the 2011 experimental plots had cysts of the “E Lindley” type, and the site used for trials in 2012 showed mixtures of the “Luffness” and “E Lindley” type. Three other fields sampled also located in East Lothian showed the presence of mixtures of Lindley and Luffness types with the predominance of the Luffness type. One cyst from field number 2 in East Lothian was not digested and two from number 3 failed to digest. Interestingly one of the cysts had the same digest pattern as the “Pa1” type (Table 4-2).

Cysts extracted from the samples taken from Shropshire gave an identical pattern to the “E Lindley” type, however two singles cyst from the Ash field and one from the Chinn field belonged to the “Luffness” type. Similar results were

obtained from the cysts collected from experimental plots in 2012 at Harper Adams. Surprisingly, the results suggest that within one cyst there was a mixture of both “E Lindley” and “Luffness” mitochondrial types.

Table 4-2 Results after digestion with a restriction enzyme TaqI of s222 PCR amplification products from field samples.

Population	RFLP result	Number of cysts
HARPER ADAMS 2011	Luffness type	3
HARPER ADAMS 2011	Lindley type	3
HARPER ADAMS 2012	Luffness type	3
HARPER ADAMS 2012	Lindley type	2
HARPER ADAMS 2012	Pa1 type	1
LUFFNESS 2011	Lindley type	6
LUFFNESS 2012	Luffness type	2
EAST LOTHIAN 1	Luffness type	5
EAST LOTHIAN 1	Lindley type	4
EAST LOTHIAN 2	Luffness type	5
EAST LOTHIAN 2	Pa1 type	1
EAST LOTHIAN 3	Luffness type	2
EAST LOTHIAN 3	Lindley type	2
CHINN	Luffness type	2
CHINN	Lindley type	6
ASH	Luffness type	1
ASH	Lindley type	2
CROWS	Luffness type	1
CROWS	Lindley type	5

4.3.2. Cytochrome B sequences

Partial sequences of the mitochondrial cytochrome B from single cysts sampled from seven fields were aligned with sequences obtained by Plantard *et al*. (2008), Pylypenko *et al.,* (2008), and The James Hutton Institute collection (unpublished). An alignment of these sequences is presented in Figure 4.2 and Figure 4.3. The differences between single cysts in their sequence polymorphisms are shown in Table 4-3. A phylogenetic tree, representing the relationships between these sequences was created and is presented in Figure 4.4 with populations of *G. mexicana* and *G. rostochiensis* used as outgroups. Populations obtained from this study were clustered into one big clade, with two subclades. In the first subclade only one sample (East Lothian 2.4) was clustered together with the Peruvian populations (Puno, Amantani, and Juliaca) and Pa1 from Scotland. The second subclade consisted of two groups and separated the Luffness and E Lindley populations. The first group consisted of three European populations, Oussant from France, the second from North of Netherlands and a third from Scotland (Luffness), which were clustered with two Peruvian populations (Arapa and Sicuani). In the second group, three cysts from Harper Adams (2011 and 2012) and from East Lothian were clustered together with European populations (Portuguese Vila real, Swiss Chavornay, French Noirmoutier Rei bois and Saint Meloir, British from Shropshire, Sacrewell Peterborough, South Scotland and Dutch from the Centre of Netherlands). The phylogenetic tree shows that in most of the sites the populations consisted of mixtures of the different mitochondrial types. Therefore this indicates that the sites used for field experiments (Chapter 3) were infested

by a mixture of population types (Luffness and E Lindley) which probably represent 2 different introductions from S. America. The field 1 from East Lothian also had two types (Lindley and Luffness) like the Luffness field in 2012. Only the E Lindley type was found in the Luffness experimental plots in 2011 whereas the East Lothian field number 2 was populated by the Pa1 or Luffness and number 3 by E Lindley.

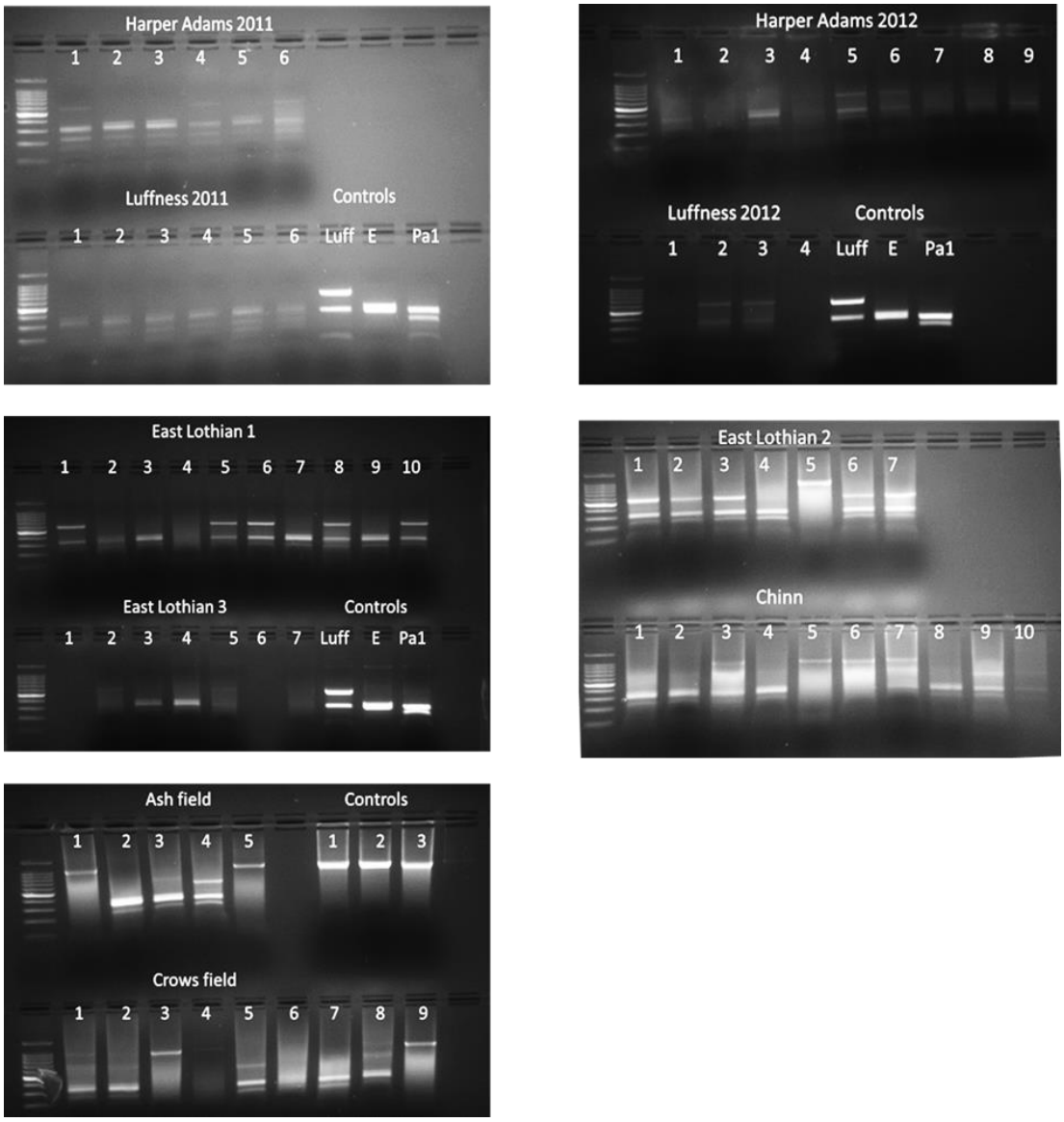

Figure 4.1 Taq1 digestion products of s222 PCR amplification products from field samples. As the controls the three clone types Luffness (Luff), E Lindley (E) and Pa1 (Pa1) were used with 100bp DNA ladder (Promega, Southampton, UK).

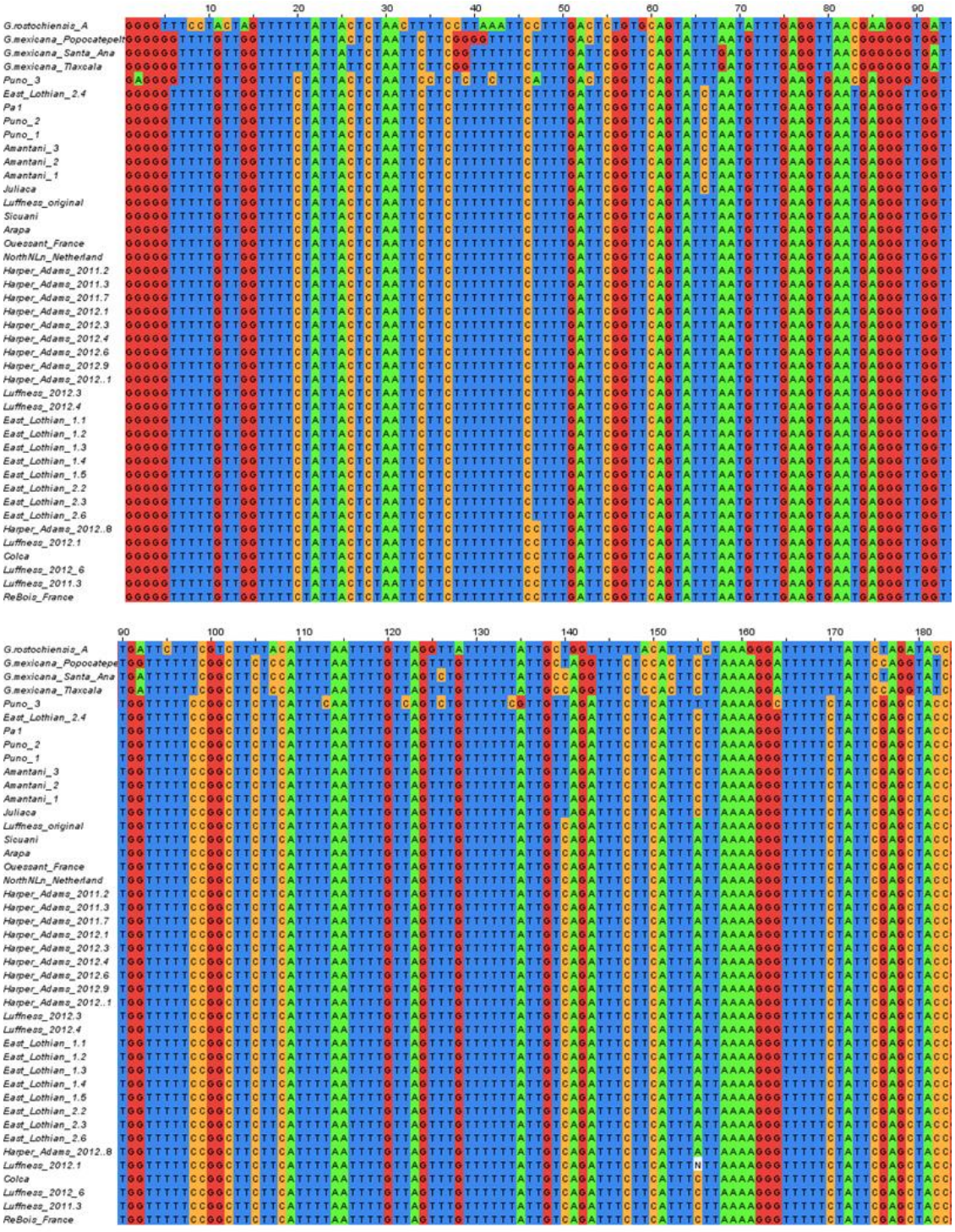

Figure 4.2 First part of the alignment of the edited sequences in Jalview (Clamp *et al*., 2004) showing the relationships between *G. pallida* Peruvian and European populations (Appendix 3) based on partial Cytochrome B sequences.

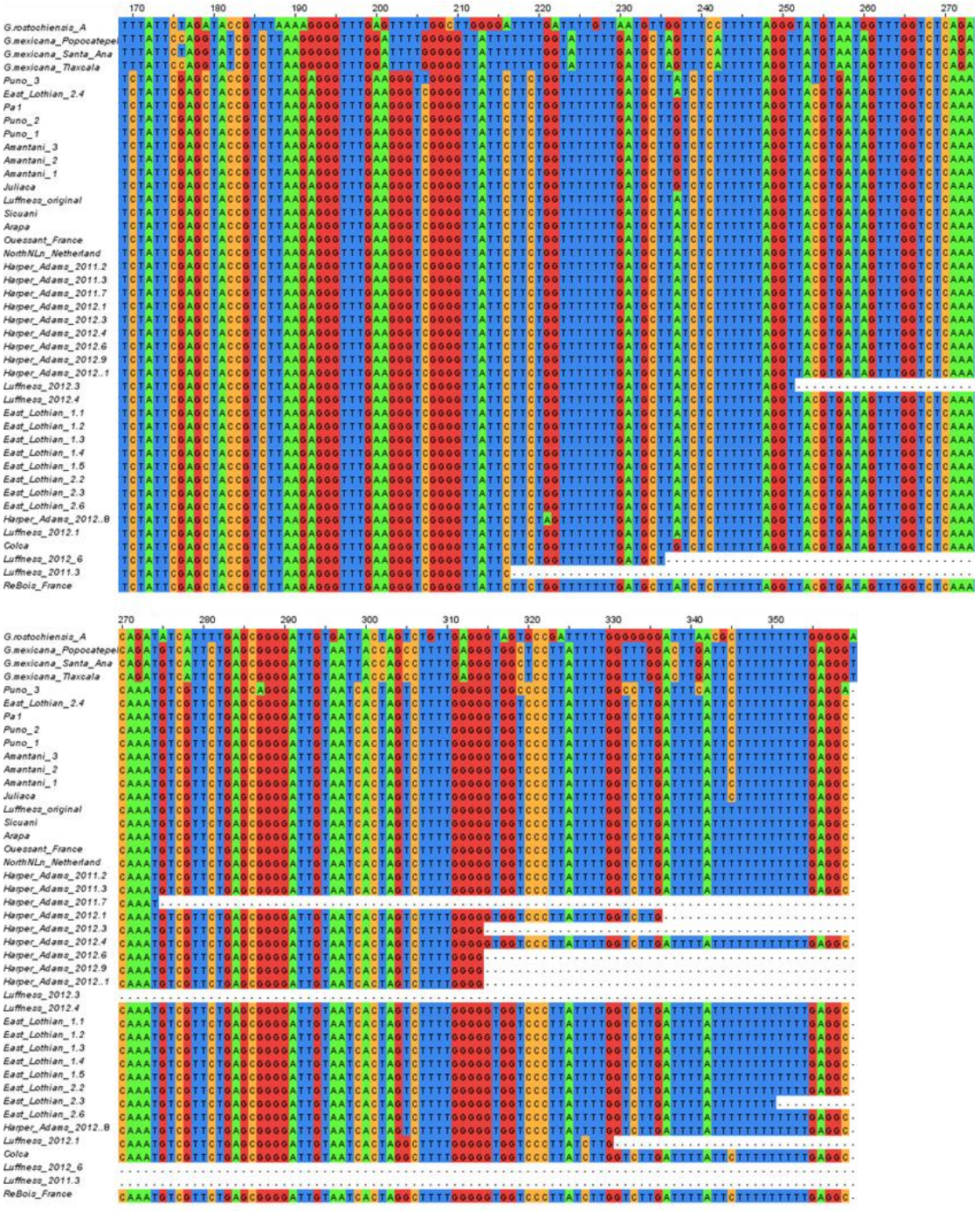

Figure 4.3 Second part of the alignment of the edited sequences in Jalview (Clamp *et al*., 2004) showing the relationships between *G. pallida* Peruvian and European populations (Appendix 3) based on partial Cytochrome B sequences.

Table 4-3 Summary of Cytochrome B sequence polymorphisms from 39 single cysts from field samples. Populations in green are the sequences that showed similarity to Luffness clone, in yellow to E Lindley and in red to Pa1. The SNP positions are indicated with reference to the sequences shown in Figure 4.2 and Figure 4.3. At position 221 both G and A were observed in the electrophenograms in some samples. Missing values are due to short sequences.

cyst	Population	47	66	140	155	221	237	305	327	345
1	HARPER ADAMS 2011.2	T	T	C	A	G (A)	A	T	T	T
2	HARPER ADAMS 2011.3	T	T	C	A	G	A	T	T	T
3	HARPER ADAMS 2011.4	C	T	C	C	G	A	G		
4	HARPER ADAMS 2011.5	C	T	C	C	G	A	G	C	C
5	HARPER ADAMS 2011.7	T	T	C	A	G	A			
6	HARPER ADAMS 2012.1	T	T	C	A	G (A)	A	T	T	
7	HARPER ADAMS 2012.2	C	T	C	C					
8	HARPER ADAMS 2012.3	T	T	C	A	G (A)	A	T		
9	HARPER ADAMS 2012.4	T	T	C	A	G (A)	A	T	T	T
10	HARPER ADAMS 2012.5	T	T	C	A					
11	HARPER ADAMS 2012.6	T	T	C	A	G (A)	A	T		
12	HARPER ADAMS 2012.7	C	T	C	C	G (A)	A			
13	HARPER ADAMS 2012.8	C	T	C	A/C	G/A	A	T	T	T
14	HARPER ADAMS 2012.9	T	T	C	A	G (A)	A	T		
15	HARPER ADAMS 2012.10	T	T	C	A	G (A)	A	T		
16	LUFFNESS 2011.3	C	T	C	C					
17	LUFFNESS 2011.6	C	T	C	C	G (A)	A	G	C	
18	LUFFNESS 2012.1	C/T	T	C	A/C	G (A)	A	G (T)		
19	LUFFNESS 2012.2	T	T	C	C					
20	LUFFNESS 2012.3	T	T	C	A	G (A)	A			
21	LUFFNESS 2012.4	T	T	C	A	G (A)	A	T	T	T
22	LUFFNESS 2012.5	C	T	C	C	G (A)	A	G		
23	LUFFNESS 2012.6	C	T	C	C	G				
24	EAST LOTHIAN 1.1	T	T	C	A	G (A)	A	T	T	T
25	EAST LOTHIAN 1.2	T	T	C	A	G (A)	A	T	T	T
26	EAST LOTHIAN 1.3	T	T	C	A	G (A)	A	T	T	T
27	EAST LOTHIAN 1.4	T	T	C	A	G (A)	A	T	T	T
28	EAST LOTHIAN 1.5	T	T	C	A	G (A)	A	T	T	T
29	EAST LOTHIAN 1.6	C	T	C	C	G (A)	A	G	C	C

30	EAST LOTHIAN 1.7	C	T	C	C	G (A)	A	G	C	C
31	EAST LOTHIAN 1.8	C	T	C	C	G (A)	A	G	C	C
32	EAST LOTHIAN 3.1	C	T	C	C	G (A)	A	G	C	C
33	EAST LOTHIAN 3.2	C	T	C	C	G (A)	A	G	C	C
34	EAST LOTHIAN 3.3	C	T	C	C	G (A)	A	G	C	C
35	EAST LOTHIAN 3.4	C	T	C	C	G (A)	A	G	C	C
36	EAST LOTHIAN 2.2	T	T	C	A	G (A)	A	T	T	T
37	EAST LOTHIAN 2.3	T	T	C	A	G (A)	A	T	T	T
38	EAST LOTHIAN 2.4	T	C	T	C	G (A)	C	T	T	C
39	EAST LOTHIAN 2.6	T	T	C	A	G (A)	A	T	T	T
	"LUFF"	T	T	C	A	G	A	T	T	T
	"PA1"	T	C	T	C	G	C	T	T	C
	"E"	C	T	C	C	G	A	G	C	C

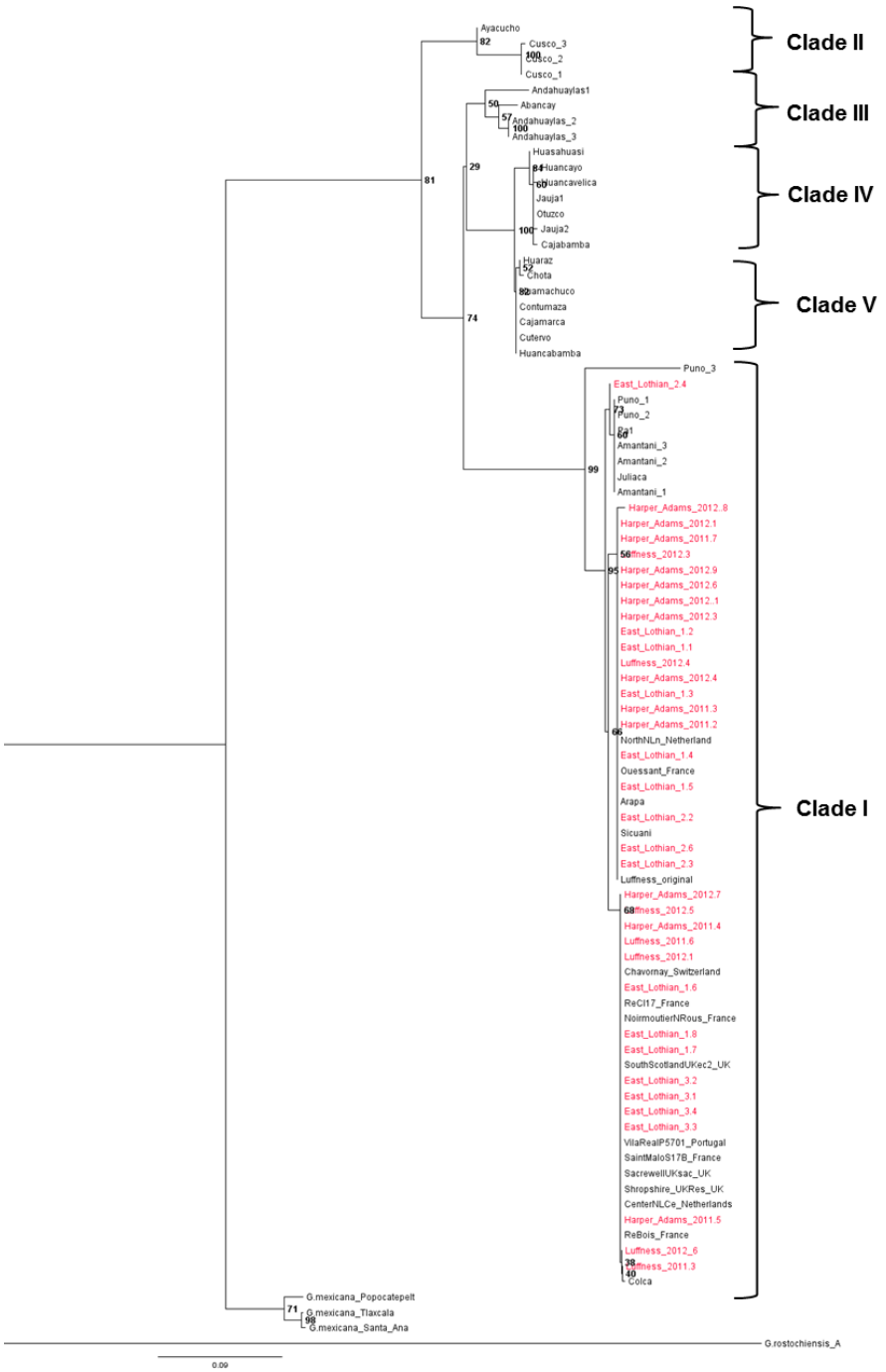

Figure 4.4 Phylogenetic tree (maximum likehood) showing the relationships between *G. pallida* populations from field samples based on partial Cytochrome B sequences with clades from Plantard *et al.* (2008). Sequences obtained from the present study are indicated in red. All sequences used for Cytochrome B analysis are included in Figure 4.2 and Figure 4.3 and Appendix 3. The numbers at the nodes are the support values from 1000 bootstraps.

4.4. Discussion

The present study was designed to molecularly characterise the populations at the field sites in Luffness and Harper Adams and to examine the composition of PCN populations in several fields near Luffness in Scotland and in England. All cysts examined belonged to one of the three European types previously described with the mitochondrial markers used. Almost all of the cysts corresponded to the E Lindley and Luffness (Pa2/3) types with 2 cysts of the Pa1 type. This finding accords with Plantard *et al.* (2008) and Pylypenko *et al.* (2008) who reported that European populations originated from southern Peru. All of the sequences from this study were clustered into clade I (Plantard *et al.,* 2008) containing the Southern Peruvian populations Puno, Amantani, Juliaca, Arapa, Sicuani and Colca which are located in the vicinity of Lake Titicaca. This also accords with observations of Pylypenko *et al.* (2008) and Grujić (2010), suggesting that the populations represented by the E Lindley and Luffness types are widespread in Europe and are likely to have originated from founder populations from S. America that are continuing to be spread within Europe, which has become a secondary distribution source. One of the most interesting findings was a cyst that clustered together with the south Peruvian populations that includes the Pa1 population. Pathotype Pa1 populations differ from Pa2/3 populations in their virulence on hosts with the *H2* resistance gene and in their isozyme profiles (Fleming and Marks, 1983). The Luffness population was also differentiated by PCR-RFLP and isozyme studies (Phillips *et al*., 1992) and is known to be more virulent than other British Pa2/3 (Phillips *et al*., 1991). The widespread occurrence of cysts with the Luffness type was also not expected.

Cysts originally collected from Luffness that are in the JHI PCN cyst collection have the Luffness type and E Lindley cysts have the E Lindley types. The finding of fields that have mixtures of these 2 types, and also the occasional presence of the Pa1 types has implications for the virulence characteristics of the field population and raises the possibility of interbreeding between these types to create hybrids with unknown virulence characteristics. The results reported in this thesis demonstrate that more than one virulence establishing protocol should be applied when investigating the populations of PCN. Comparison of the digestion results of the noncoding scmtDNA IV with the phylogenetic analyses of the cytochrome B revealed that both methods successfully determined the same populations of *G. pallida*: E Lindley, Pa1 and Luffness. Due to the limited replication (only ten cyst per field were tested) variations within the fields might not have been detected by a single method, however a combined data set of both scmtDNA and CytB markers gave a better perspective of the virulence of analysed populations.

5. SUMMARY AND CONCLUSIONS

Potato cyst nematodes (PCN) are major parasites of potato and other members of the *Solanaceae* family. PCN causes substaintial crop yield loss to world-wide agriculture. Infected plants usually have a smaller root system, which explores a smaller volume of soil (Trudgill, 1983) and the damaged roots are adversely affected by water stress and disturbances of nutrient metabolism (see Chapter 1). Generally, the population of *Globodera* spp. in the field at the time of planting correlates with the level of yield losses (Chapter 3). The population dynamics of PCN and related yield loss of the potato crop, are dependent on the initial population of PCN in the field as well as environmental factors, soil type, and cultivar tolerance and resistance.

This thesis reports the results of experiments conducted on the life cycle of PCN under laboratory (Chapter 2) and the field conditions (Chapter 3) to obtain a better understanding of factors that affect population dynamics of PCN in different agroecological conditions. The initial aim was to determine the duration of the life cycle of PCN and the number of generations per year in different temperature regimes. Establishing the total hatch in potato root diffusate (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 (*Globodera pallida* and *G. rostochiensis*). The species significantly differ in hatching and temperature preferences. These differences in the hatching responses have implications for the rates and amounts of multiplication and competition between the two species in particular soil

temperature profiles. *G. pallida* was more efficient in overall hatching, whereas *G. rostochiensis* generally hatched more quickly. Also, *G. pallida* had a broader temperature range over which similar amounts of hatching occurred and low soil temperatures are likely to favour *G. pallida,* whereas warmer temperatures are likely to favour *G. rostochiensis*. The results also suggest that the hatching response for both species of PCN is greater and faster at the higher temperatures tested and therefore increases in soil temperatures due to regional climatic differences or climate change are likely to favour PCN multiplication.

Motile juveniles and adult life stage PCN were examined for their response to temperature variation. Chapter 2 describes a series of experiments, which were carried out in pots in semi natural conditions. The occurrence of juveniles in the soil reflects the time period, amount of hatching that has occurred in the different temperature regimes and also indicated whether a second hatch had occurred. At higher soil temperatures the amount and the speed of population multiplication was greater. In the growth cabinet experiments the presence of a second peak of juveniles indicates that diapause was not obligatory in these conditions. The results from Chapter 2 and Chapter 3 suggest that one generation of *G. pallida* takes <10 weeks at average soil temperatures found in UK and therefore it is possible that 2 generations could be completed in <20 weeks at warmer temperatures. The experiment examining the emergence of females at different temperatures and on different cultivars revealed also the response in the number of females observed. The most optimal temperature for the development of females was 16°C on the susceptible cv Desirée, with the

first appearance 4 weeks after inoculation. Although, the numbers of females observed was greatly reduced with the partially resistant cultivar Vales Everest, there was still an effect on the response at different temperatures. The difference in occurrence of the first males (5 weeks) and females (4 weeks) could be due to the less efficient recovery of males from the soil.

The field experiments were undertaken to compare and relate the results from the life cycle experiments performed in controlled conditions to those in naturally infested potato fields. The trials were located in 2 different agroecosystems with different soil temperature profiles.

The findings observed in the field trials support the results from those in laboratory conditions, and support the hypothesis that parts of the UK with higher soil temperatures, or years in which crop planting coincides with warmer soil temperatures, are likely to have higher levels of hatching of PCN and thus greater multiplication. The presence of juveniles inside the stained root samples at the end of the growing season might support the possibility that the diapause stage can be omitted leading to a second hatch and consequently a second generation of PCN in the fields with warmer soil temperatures. This might lead to greater challenges in controlling population levels through use of nematicides and rotation, and in limiting spread. However, if the timing of harvest is correct the second hatch might be an opportunity to decrease the population in the field. These experiments, which provide data from which the impact of temperature on PCN population dynamics can be predicted, should assist growers in making appropriate management decisions for their particular circumstances.

Although a small number of field trials were conducted in this study, the findings suggest that the nematicide treatments were not able to prevent all hatching or root invasion by juveniles in field conditions, but that, they delayed these processes significantly. Woods *et al*., (1999) came to the conclusion that fosthiazate inhibits hatching temporarily of *G. pallida* in an *in vitro* test and delayed hatching in soil. Results from the Harper Adams 2011 field trail supported this hypothesis, emphasising the temporality of the hatch inhibition. This indicates the importance of the harvest time, for example at the end of field experiments there was no difference in reproduction factor between treated with nematicide and untreated experimental plots. At the Luffness (2011) field site, the Vydate (oxamyl) treatment reduced the population significantly. However, it is hard to determine whether the difference between a reduction in the multiplication rate is caused by the nematicide application or due to the higher initial number of eggs in the soil. Low initial densities and cold- wet weather in 2012 made it difficult to reveal differences between the cultivars and nematicide treatment. At Harper Adams the multiplication rate was extremely high on the untreated fields. At both sites Vydate was used as a control method, and there was no difference in multiplication rates between the nematicide and non-nematicide treated plots. In both treatments the Pf/Pi rate was significantly higher at Harper Adams than at Luffness, which might be related to the low densities and poor environmental conditions, as well as differences in the virulence between two populations of *G. pallida* (Phillips *et al.,* 1991; Trudgill *et al.,* 2014).

The field experiments in Chapter 3 also showed that there is a strong relationship between PCN multiplication and initial density (Pi), moreover indicated that Pi is negatively correlated with the reproduction rate of PCN. The results also suggest that the lower initial population correlates with a higher reproduction rate. Therefore low densities of PCN might significantly increase crop losses in subsequal planting years. This finding also has important implications for modelling PCN population dynamics for use in establishing control methods.

The results in Chapters 2 and 3 confirmed that resistant and partially resistant cultivars are effective in suppressing multiplication of PCN and thus are important control methods. The cultivar Vales Everest greatly stopped population multiplication of *G. pallida* in the laboratory experiments (female canister experiment and competition experiment), and in the field experiments slowed down multiplication of PCN in the plots resulting in the lowest Pf/Pi rate compared to other cultivars at Harper Adams.

Furthermore, Chapter 3 included an investigation of the relationship between the population dynamics of PCN in terms of species composition and initial density in the field. The results showed a significant reduction in the multiplication rate of both species in pots inoculated with mixed populations.

A decrease in *G. rostochiensis* multiplication rate was found when *G. pallida* was present in higher or equal initial densities on cultivars Desirée and Vales Everest.

One explanation is that *G. pallida* hatched more efficiently thereby allowing this species to occupy the roots more effectively and reducing available sites for *G.*

rostochiensis development. The opposite effect was observed on Vales Everest when the main species in the inoculum was *G. rostochiensis.* Partial resistance combined with higher density of *G. rostochiensis* reduced multiplication rate of *G. pallida*, implying that there could be feeding site competition between the 2 species.

In chapter 4 molecular markers were used to characterise populations of PCN in the field trial sites and in other potato fields in the 2 regions. Two mitochondrial DNA markers (s222 and partial Cytochrome B) were used to characterise individual cysts. The majority of examined cysts were classified as *G. pallida* pathotype Pa2/3 which is consistent with these populations belonging to the same molecular groups as those of the majority of the other European populations that have been characterised, and indicates that they could have resulted from the continuing spread of *G. pallida* within Europe. In one of the East Lothian fields and in one from Shropshire, Pa1 cysts were found, which indicates that this pathotype is not restricted to Duddingston, Scotland as described (Stone *et al.,* 1986). The presence of Pa1 and Pa2/3 molecular types within the same field raises the potential for interbreeding between a populations and the possibility of novel virulence characteristics.

The work presented in this thesis is the first example of the application of quantitative PCR to investigate population dynamics of PCN in field conditions and as reported in detail in the results section in Chapter 3, the application of qPCR provided less variable results. Both of these methods were combined to investigate the life cycle and the population dynamics of PCN from two field

sites in the United Kingdom where PCN were naturally present,The results of the application of qPCR provided less variable results compared to visual analysis of the root infestation. The ability to detect the nematode infestation in the field using qPCR is an important step forward which provides a more reliable estimate of the level of infestation, and can help monitor the results of control of PCN population.

Due to limited resources and space, technical replication was not at a satisfactory level (for the growth cabinet experiments in Chapter 2). This meant that it was not possible to obtain the significant statistical power and the statistical analysis could not be performed. A second limitation was the dependence on natural PCN infestation within the fields chosen for the field trails, as shown in Chapter 3. The populations of PCN varied between year and locations. For example, in 2012 infestations of the potato fields were extremely low, which made it hard to investigate the population dynamics of PCN in this year.

Nevertheless, in conclusion warmer soil temperatures increase population levels on susceptible hosts and increase damage to the crop. 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 greater multiplication and have greater challenges in controlling population levels. Once introduced to the field PCN is difficult to control, mainly due to of the lack of fully resistant cultivars (*G. pallida*) and the fact that nematicides are at risk of complete withdrawn from the market due to their hazardous nature. The presence of different pathotypes for each potato cyst nematode species

complicates further the control of PCN as some pathotypes are more virulent than others. When the species composition in the field is known, appropriate control method can be employed. For successful control of PCN a combination of the timing of the harvest to limit population multiplication, use of resistant varieties and crop rotation with limited nematicide application can be used in integrated pest management strategies. Knowledge of this background variation allows a more precise assessment of the performance of various PCN control measures in different environments and application of low risk control strategies.

6. **FUTURE WORK**

The data obtained in this thesis could be used for the further development of the PC PCN integrated pest management system as a selection and timing tool. The data is also being used for the development of a dynamic stage-structured simulation model for PCN based on time delay differential equations with determination by climate parameters. The model is designed to study the dynamics of various life stages (egg-juvenile-adult) using the data obtained in this study.

Chapter 2 and Chapter 3 indicated that there are significant differences in the population dynamics, yield loss due to differences in soil temperature between seasons, and species-specific temperature responses in development, all of which the current PC PCN model does not describe. Combining the dynamic model of PCN population development from hatching to formation of eggs in new cysts, including the possibility of second generation within one growing season, with the model of potato crop growth from planting to harvest would broadly predict the mechanisms of yield reduction and population increase.

Improving assays for pathotyping PCN is also worth further investigation, as pathotype differentiation is important for choosing the best potato cultivar with tolerance and resistance against potato cyst nematodes. In this research, molecular techniques of sequence comparison of cytochrome B and PCR RFLP were used to examine the intraspecific composition of the PCN in field samples. These tools combined with other diagnostic methods for species determination, could provide additional information about the intra-specific characteristics of

potato cyst nematodes populations but they still require validation to show that the molecular markers are associated with specific phenotypes.

Finally, taken together, the opportunity for developing better tools for PCN management presented above, i.e.: updating the PCN model and improving pathotyping methods that would allow control strategies that are most appropriate for a certain region and agroecological situation to be employed. This will be beneficial for future intergrated pest management approaches used within sustainable agriculture strategies. Further work needs to be done to establish the role of temperature on competition between the two species and how this effects competition at feeding sites directly in the roots in different potato genotypes. Inter and intra-specific competition and the impact of temperature on decline rates merit further investigation to assess whether these variables should be included in the PCN population dynamics model.

Another area that would be interesting to investigate in the future is the second generation nematodes that were able to avoid entry into diapause. Chapter 2 and 3 indicated that in suitable conditions PCN is able to avoid the diapause stage. A comparison of these nematodes with those that do enter diapause could help identify which genes are responsible for the diapause stage and what triggers them.

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8. **APPENDICES**

Appendix 1

```
#cs ----------------------------------------------------------------------------

 AutoIt Version: 3.3.6.1
 Author:         Sebastian Eves-van den Akker
 Script Function: Measure Cyst size


#ce ----------------------------------------------------------------------------
Global $Paused
Global $test
HotKeySet("{PAUSE}", "TogglePause")
HotKeySet("{ESC}", "Terminate")
HotKeySet("{PGUP}", "Stoploop")


Func TogglePause()
  $Paused = NOT $Paused
  While $Paused
    sleep(100)
    ToolTip('Script is "Paused"',0,0)
  WEnd
  ToolTip("")
EndFunc

$count = 0
#Include <Misc.au3>
#include <Array.au3>
#include <File.au3>
#include <GUIConstantsEx.au3>
#include <SliderConstants.au3>
#Include <GUIConstantsEx.au3>
#Include <WindowsConstants.au3>
#Include <WinAPI.au3>
#include <ScreenCapture.au3>
;===============================================================
=============================================

_DwmEnable(False)
;====================Creates the Graphical User Interface of the
Program================================
GUICreate("Colour Counter", 240,320,@DesktopWidth -245,0)
```

```
$next = GUICtrlCreateButton("Start!",20, 10, 80, 20)
$colorchange = GUICtrlCreateButton("Colour change",120, 10, 80, 20)
$slider1 = GUICtrlCreateSlider(10, 50, 220, 20,$TBS_TOP)
GUICtrlSetLimit(-1, 200, 1)
$button = GUICtrlCreateInput("Shades of Variation",175, 70, 50, 20)
$n3 = GUICtrlCreateInput("#",20, 120, 50, 20)
GUICtrlCreateGroup ( "Shades of Variation", 10, 35, 220,60)
GUICtrlCreateGroup ( "Pixel Count", 10, 100, 70,45)
$check = GUICtrlCreateCheckbox("Reporter", 100, 100, 120, 20)
$checkgfp = GUICtrlCreateCheckbox("Background Agata", 100, 130, 120, 20)
GuiCtrlSetState($checkgfp, $GUI_CHECKED)

$button5 = GUICtrlCreateInput("Colour",15, 170, 105, 20)
GUICtrlCreateGroup ( "Malual input colour", 10, 150, 120,50)
$checkmanual = GUICtrlCreateCheckbox("Manual Colour", 140, 170, 120, 20)

GUICtrlCreateGroup ( "Relative size", 10, 205, 220,100)
$pixpermm = GUICtrlCreateInput("pixel per mm",15,220,90, 20)
$n4 = GUICtrlCreateInput("#",120, 220, 100, 20)
$numberofnematodes= GUICtrlCreateInput("# of cysts",15,250,90, 20)
$calc = GUICtrlCreateButton("Calculate",120, 250, 60, 20)
$pernema= GUICtrlCreateInput("average size",15,280,150, 20)
 GUISetState()
;=====================================================
==============================================
Fileread("Colour Counter.txt")
if @error then
_Filecreate("Colour Counter.txt")
endif
;=====================================Default      Shades      of
variation, 42==================================
GUICtrlSetData($button, 150)
GUICtrlSetData($slider1, 150)


Do
        $msg = GUIGetMsg()

        if $msg = $GUI_EVENT_CLOSE Then
                exit 0
        endif

$sliderread = GUICtrlRead($slider1, 1)
GUICtrlSetData($button, $sliderread)

        if $msg = $GUI_EVENT_CLOSE Then
```

```
		exit 0
		endif

tooltip("Click Start when ready to start",0,0, "If at any time you wish to end Press ESC or Press PAUSE-BREAK to pause")



if $msg = $colorchange Then
global $date = @MDAY & "/" & @MON & "/" & @YEAR
global $time = @HOUR & "." & @MIN & "." & @SEC
	GuiCtrlSetState($checkgfp, $GUI_UNCHECKED)
	do
tooltip("click on new colour",0,0)
sleep(50)
until _ispressed(01)
$array = mousegetpos()
$color = "0x" & hex(pixelgetcolor ($array[0],$array[1]),6)
Filewriteline("Colour Counter.txt",">" & $color &"    Date:"& $date & "     Time:" & $time )
sleep(500)
	endif

if GUIctrlread($checkmanual) = $GUI_CHECKED then
	GuiCtrlSetState($checkgfp, $GUI_UNCHECKED)
	$color = GUIctrlread ($button5)
endif


	if $msg = $calc Then

		$numberofnematodesx = guictrlread($numberofnematodes)
		$n4x = guictrlread($n4)

		guictrlsetdata ($pernema, round ($n4x/ $numberofnematodesx ,3) & "mm2 per cyst")
		;calc per nema

		EndIf



if $msg = $next and (GUIctrlread($checkmanual) = $GUI_CHECKED or GUIctrlread($checkgfp) = $GUI_CHECKED)  Then

sleep(500)
do
```

```
do

tooltip("click top left",0,0)
sleep(50)
until _ispressed(01)
$array = mousegetpos()
sleep(500)
do

tooltip("click bottom right",0,0)
sleep(50)
until _ispressed(01)
$array1 = mousegetpos()

$y = $array[1]
$x = $array[0]
$xb = $array1[0]
$yb = $array1[1]

$shades = GUIctrlread($slider1)

$xta = $x
$yta = $y
$xba = $xb
$yba = $yb
$time = timerinit ()
tooltip("working, wait",0,0)

if GUIctrlread($checkgfp) = $GUI_CHECKED then
$color = 0xFEFEFE ;white ish bit
endif


$color2 = 0x000000
$hDC = _WinAPI_GetWindowDC(0)

do


Do
        $x = $x + 1
        $pix = pixelsearch($x,$y,$x+1,$y+1,$color,$shades)
        if @error then
                $count = $count + 1
                if GUIctrlread($check) = $GUI_CHECKED then
                        _WinAPI_DrawLine($hDC,$x,$y,$x+1,$y)
                endif
```

```
        endif

until $x > $xba
$x = $xta
$y = $y + 1
until $y > $yba
tooltip("done",0,0)
$diff2 = timerdiff($time)


$pixread = guictrlread($pixpermm)


GUIctrlsetdata($n3,$count)

GUIctrlsetdata($n4,round ($count/($pixread*$pixread),3) & " mm2")
$date = @MDAY & "/" & @MON & "/" & @YEAR
$time = @HOUR & "." & @MIN & "." & @SEC
Filewrite ("agatadata.txt", "Date:"& $date & "      Time:" & $time & " size = " &
$count & @CRLF)
Filewrite ("agatadata.txt", $count & @CRLF)
controlsend("Microsoft Excel - Book1", "", "",$count & @CRLF)
$count = 0

tooltip("",0,0)
sleep(100)
until _ispressed (31)
endif


        until $msg = $GUI_EVENT_CLOSE

        Func _DwmEnable($WhatToDo)
DllCall("dwmapi.dll", "long", "DwmEnableComposition", "uint", $WhatToDo)
EndFunc
```

Appendix 2

Table 8-1 Minimum, maximum and mean temperatures in °C recorded in 20cm depth in potato ridges with DS1920-F5 Temperature ibuttons (HomeChip, Milton Keynes, UK) together with planting and harvesting dates of potato crops.

Year	Site (Country)	Dates of planting and harvesting potatoes	Minimum temperature [°C]	Maximum temperature [°C]	Mean temperature [°C]
2010	Luffness Millfield (Scotland)	27/05-11/10	11.5	22.5	17.01
	Luffness Forefield (Scotland)	27/05-11/10	10.5	21.5	15.63
2011	Whitewater (Scotland)	19/05-14/09	8	18.5	13.21
	Balruddery (Scotland)	19/05-27/09	9	20	13.62
	Luffness (Scotland)	4/5—28/09	5	28.5	14.12
	Harper Adams (England)	21/04-21/09	9.5	22.5	15.38
	Ross-on-Wye (England)	18/04-20/08	11	19	15.26
	Leominster (England)	19/04-22/08	9.5	31.5	14.8
	Tetbury (England)	14/04-02/08	9.5	25	15.05
	Kings Caple (England)	14/04-23/08	8.5	27.5	16.07
	Bold Farm (England)	31/05-26/09	11.5	26	15.51
2012	Elgin (Scotland)	16/05-20/09	6.566	24.59	14.48
	Harper Adams (England)	02/04-04/10	4.5	36.5	13.67
	Roadside (England)	03/05-13/08	6.555	23.6	15.53
	Balruddery (Scotland)	17/05-23/09	2.5	30	13.16
2013	Howell field (England)	13/04-19/09	6	31.5	15.12
	High Pilmore (Scotland)	29/05-26/09	9.567	24.6	16.11
	Whitewater (Scotland)	21/05-16/09	6	27.5	15.81
	Legge (England)	09/05-23/09	4.5	31	15.9
	Elgin (Scotland)	22/05-14/10	5.062	26.09	14.42
	Ayr	04/06-10/10	8	25	15.21

Table 8-2 Grid references of the fields with monitored soil temperatures.

Site	Latitude	Longitude
Tetbury	51.665	-2.275718
Leominster	52.305	-2.818766
Ross on Wye	51.892	-2.568096
King Caple	51.965	-2.624453
Luffness	56.008	-2.830929
Whitewater	56.729	-2.899611
Harper	52.78	-2.425381
Balruddery	56.477	-3.114739
Howell	53.001	-0.289299
Legge	52.534	0.434622
Elgin	57.661	-3.449282
Bold Farm Robin Cropper	53.545	-2.876695
Roadside	52.819	0.107189

Appendix 3

Table 8-3 Populations of PCN used for Cytochrome B analysis described in Chapter 4.

	Population/strain	Country of origin/grid coordinates	Sequence source cyt B	PCR-RFLP test
1	East Lothian Field 1	Scotland (East Lothian)	This study	This study
2	East Lothian Field 2	Scotland (East Lothian)	This study	This study
3	East Lothian Field 3	Scotland (East Lothian)	This study	This study
4	Luffness 2011	Scotland (East Lothian) 56.007963, -2.830929722	This study	This study
5	Luffness 2012	Scotland (East Lothian)	This study	This study
6	Harper Adams 2011	England (Shropshire) 52.809072, -2.460127	This study	This study
7	Harper Adams 2012	England (Shropshire) 52.78, -2.425381	This study	This study
8	Chinn field	England (Herefordshire) 51.87918, -2.61024	Not tested	This study
9	Crows Field	England (Shropshire) 52.76220, -2.48893	Not tested	This study
10	Ash Field	England (Shropshire) 52.73867, -2.33200	Not tested	This study
11	Abancay	Peru	(Plantard *et al*., 2008)	Not tested
12	Amantani_1	Peru	(Plantard *et al*., 2008)	Not tested
13	Amantani_2	Peru	(Plantard *et al*., 2008)	Not tested
14	Amantani_3	Peru	(Plantard *et al*., 2008)	Not tested
15	Andahuaylas_2	Peru	(Plantard *et al*., 2008)	Not tested
16	Andahuaylas_3	Peru	(Plantard *et al*., 2008)	Not tested
17	Andahuaylas1	Peru	(Plantard *et al*., 2008)	Not tested
18	Arapa	Peru	(Plantard *et al*., 2008)	Not tested
19	Ayacucho	Peru	(Plantard *et al*., 2008)	Not tested
20	Cajabamba	Peru	(Plantard *et al*., 2008)	Not tested
21	Cajamarca	Peru	(Plantard *et al*., 2008)	Not tested
22	CenterNLCe	Netherlands	(Plantard *et al*., 2008)	Not tested
23	Chavornay	Switzerland	(Plantard *et al*., 2008)	Not tested
24	Chota	Peru	(Plantard *et al*., 2008)	Not tested
25	Colca	Peru	(Plantard *et al*., 2008)	Not tested
26	Contumaza	Peru	(Plantard *et al*., 2008)	Not tested

27	Cusco_1	Peru	(Plantard *et al*., 2008)	Not tested
28	Cusco_2	Peru	(Plantard *et al*., 2008)	Not tested
29	Cusco_3	Peru	(Plantard *et al*., 2008)	Not tested
30	Cutervo	Peru	(Plantard *et al*., 2008)	Not tested
31	*G. mexicana*_Popocatepetl	Peru	(Plantard *et al*., 2008)	Not tested
32	*G. mexicana*_Santa_Ana	Peru	(Plantard *et al*., 2008)	Not tested
33	*G. mexicana*_Tlaxcala	Peru	(Plantard *et al*., 2008)	Not tested
34	*G. rostochiensis*_A	Scotland	JHI	Not tested
35	Huamachuco	Peru	(Plantard *et al*., 2008)	Not tested
36	Huancabamba	Peru	(Plantard *et al*., 2008)	Not tested
37	Huancavelica	Peru	(Plantard *et al*., 2008)	Not tested
38	Huancayo	Peru	(Plantard *et al*., 2008)	Not tested
39	Huaraz	Peru	(Plantard *et al*., 2008)	Not tested
40	Huasahuasi	Peru	(Plantard *et al*., 2008)	Not tested
41	Jauja1	Peru	(Plantard *et al*., 2008)	Not tested
42	Jauja2	Peru	(Plantard *et al*., 2008)	Not tested
43	Juliaca	Peru	(Plantard *et al*., 2008)	Not tested
44	Luffness_original	Scotland	JHI	Not tested
45	NoirmoutierNRous	France	(Plantard *et al*., 2008)	Not tested
46	NorthNLn	Netherland	(Plantard *et al*., 2008)	Not tested
47	Otuzco	Peru	(Plantard *et al*., 2008)	Not tested
48	Ouessant	France	(Plantard *et al*., 2008)	Not tested
49	Pa1		(Plantard *et al*., 2008)	Not tested
50	Puno_1	Peru	(Plantard *et al*., 2008)	Not tested
51	Puno_2	Peru	(Plantard *et al*., 2008)	Not tested
52	Puno_3	Peru	(Plantard *et al*., 2008)	Not tested
53	ReBois_France	France	(Plantard *et al*., 2008)	Not tested
54	ReCl17_France	France	(Plantard *et al*., 2008)	Not tested
55	SacrewellUKsac	England	(Plantard *et al*., 2008)	Not tested
56	SaintMaloS17	France	(Plantard *et al*., 2008)	Not tested
57	Shropshire_UKRes	England	(Plantard *et al*., 2008)	Not tested
58	Sicuani	Peru	(Plantard *et al*., 2008)	Not tested
59	SouthScotlandUKec2	Scotland	(Plantard *et al*., 2008)	Not tested
60	VilaRealP5701	Portugal	(Plantard *et al*., 2008)	Not tested