Summary Report

Durability of resistance against *Globodera pallida*

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1. SUMMARY

- Virulence against the most commercially widely used resistance sources can be selected if PCN is repeatedly exposed to these resistance sources
- This increased virulence is specific against the resistance source used for selection rather than representing a shift in general aggressiveness of PCN
- Combining a range of different resistance sources in a single cultivar will therefore provide more durable resistance that will be extremely difficult for PCN to overcome
- A new genome assembly for *Globodera pallida* has been generated that shows greatly improved metrics compared to the published sequence
- Candidate avirulence genes for H3 and *S. vernei* resistance sources have been identified for functional testing
- A system for functional testing of candidate avirulence genes has been developed

2. INTRODUCTION, METHODS AND KEY RESULTS, CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

Introduction

The potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* continue to cause damage to the UK potato crop. Control options for these nematodes are becoming more limited due to removal of many effective nematicides due to environmental concerns. Natural resistance is the most cost effective and environmentally friendly way of controlling PCN. However, fully resistant varieties have been developed only against *G. rostochiensis* (*H1*-containing varieties). Continuous use of these cultivars has led to strong selection in favour of *G. pallida* (Whitehead and Turner, 1998), and this is now the predominant species in many UK potato fields. Resistance against *G. pallida* is more difficult to find, due to the complex nature of the *G. pallida* presence in European soils. It is thought that the initial introduction of *G. pallida* was more genetically heterogenous than that of *G. rostochiensis*, meaning that whereas the vast majority of the *G. rostochiensis* present in the UK is of a single pathotype (Ro1) that is controlled by H1, there are several different pathotypes of *G. pallida* present. Three main pathotypes of *G. pallida* have been recognised in Europe: Pa1, Pa2 and Pa3, according to the Kort et al. (1977) classifications. Sequencing of the *cyt b* mitochondrial gene has allowed profiling of the *G. pallida* present in Scotland. This analysis has shown that fields regularly contain a mixture of different genotypes of this nematode (Eves-van den Akker *et al.,* 2015). This genetic diversity presents a potential problem in terms of management of resistance as there is clearly the potential for selection of virulent nematodes from a mixed population if partially resistant cultivars are repeatedly grown.

Resistance breeding efforts for *G. pallida* have focused on two principal resistance sources. The H3 resistance source is derived from *Solanum tuberosum ssp. andigena* CPC2802 and is present in the cultivar Vales Everest. However, the most widely used source of *G. pallida* resistance is termed *Gpa5* and derives from *S. vernei.* It should be noted that a range of *S. vernei* accessions have been used to introgress resistance into potato varieties or breeding clones including the breeding clone LGU8 (from USA), the European clones V24/20 (from Germany) and VRN1-3 (from the Netherlands) (Rigney et al., 2017; van Eck et al., 2017). Consequently different populations of the same *G. pallida* pathotype may display different virulence ranges on resistance from *S. vernei* depending on the background of each source. *Gpa5* is the resistance source present in the cultivars Innovator and Arsenal.

Plants have a multi-layered immune system to protect themselves from attack by pathogens. The evolution and function of the plant immune system has been summarised in the Zig-Zag model (Jones & Dangl, 2006). The majority of potential pathogens are detected due to the presence of conserved pathogen molecules (Pathogen Associated Molecular Patterns – PAMPs) activating the first layer of defence responses, Pattern Triggered Immunity (PTI). Successful biotrophic pathogens are able to deploy effector proteins that suppress PTI and allow the pathogen to infect. In the second layer of plant defences, the products of resistance genes recognise the presence of a specific effector and activate a strong resistance response,

often involving a localised cell death known as the hypersensitive reaction. An effector recognised by a resistance gene is termed an **avirulence gene**. Pathogens that overcome resistance often do so by making modifications to the recognised avirulence gene; these modifications may include removal of the recognised gene or minor changes to the sequence that allow it to function while evading recognition.

As a result of genome and transcriptome projects for PCN (Cotton *et al.,* 2014; Eves-van den Akker *et al.,* 2016) full lists of several hundred candidate effectors have been identified from these species. In addition, previous work at The James Hutton Institute has allowed populations of *G. pallida* that have been grown repeatedly on potato cultivars containing H3 or *Gpa5* to be generated. These selected lines show increased virulence against these resistance sources (Phillips and Blok, 2008).

Although the breeding programmes described above have allowed cultivars with good levels of resistance against *G. pallida* to be generated, it is not clear how durable the resistance will be. There are already reports of *G. pallida* populations in continental Europe that overcome the *S. verneii Gpa5* resistance present in *Innovator* (G. Smant, Wageningen University, unpublished). Knowledge of which effectors are recognised by H3 and *Gpa5* would allow the likely durability of these resistance sources in the UK to be determined: substantial variation in the recognised effector within UK *G. pallida* populations would suggest that selection for a non-recognised form is likely whereas resistance that recognises a highly conserved effector is likely to be more durable. The **aims** of this project were therefore:

1. To examine the virulence of lines of *G. pallida* selected on potato cultivars containing various resistance sources in order to determine the potential for selection of virulent *G. pallida*

2. To use capture enrichment sequencing of effector-encoding genes of *G. pallida* from avirulent and virulent populations of nematodes in order to identify potential candidate avirulence genes

3. To develop a system for functional testing of candidate avirulence genes

Methods and Key Results

1. Selection for virulence in field populations of G. pallida

Two English *G. pallida* field populations (Newton and Farcet) and derived sub-populations selected on various resistance sources were used. These "founder" populations had been reared on four partially resistant potato clones for 9 generations (Phillips and Blok, 2008); two of them derived from *Solanum vernei* (clones Sv_8906 (Guardian) and Sv_11305 (Morag_) and two from *S. tuberosum* ssp. *andigena* CPC2802 (clones Sa_11415 and Sa_12674). Subsequently, the Newton (n) selected populations were called n-8906, n-11305, n-11415 and n-12674, while the Farcet (f) selected populations f-8906, f-11305, f-11415 and f-12674 respectively (Figure 1).

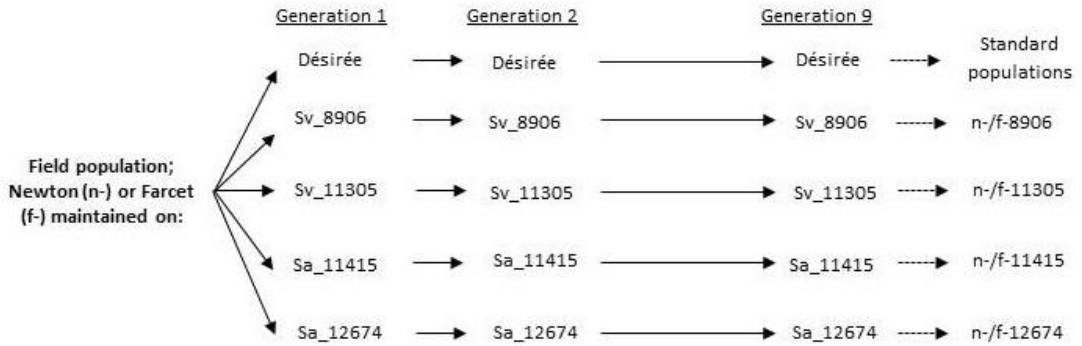

***Figure 1**: Populations of G. pallida used for virulence analysis*

Nine potato genotypes were screened in virulence tests. Five of these were commercial varieties (Désirée, Vales Everest, Innovator, Royal and Arsenal) and 4 were breeding clones from The James Hutton Institute (Sv_8906, Sv_11305, Sa_11415 and Sa_12674). The Sv_8906 and Sv_11305 lines were used for developing the varieties Guardian and Morag respectively (Phillips and Blok, 2008). The resistance sources in these potato lines were derived from either *S. vernei* or *S. tuberosum ssp. andigena* CPC2802, except for the susceptible cultivar Désirée and Royal. Royal (parentage Midas x 92-BUY-1) is a Danish variety that is highly resistant to *G. rostochiensis* Ro1 but is susceptible to all the *G. pallida* pathotypes.

The tests were carried out in root-trainer pots (12cm deep) (Haxnicks). These were three-quarters filled with insecticide-free compost containing 20 cysts. A tuber section (sized ≈ 1.5cm x 1.5cm), from each potato genotype, containing a single sprouted eye was planted on the surface of the compost with the sprout pointing downwards. Each experiment was repeated twice and each 'nematode population x potato line' combination had 4 replicates in each complete randomised experiment (Figure 2). All the cysts had previously been tested for vitality and fungus contamination in hatching tests.

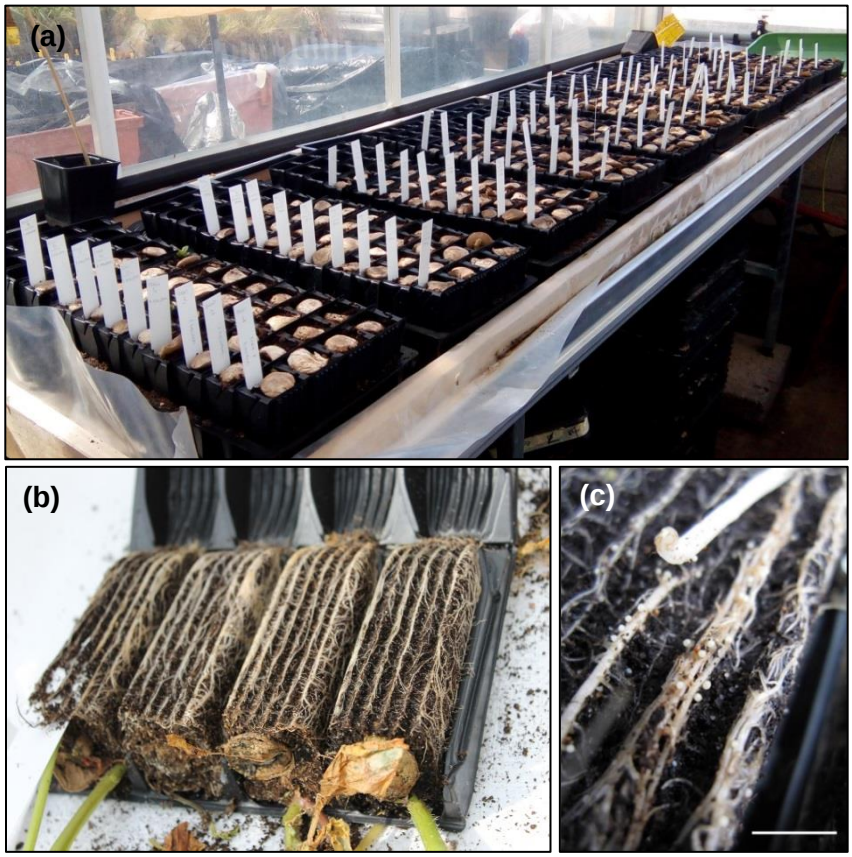

Figure 2: *Set up of virulence tests. (a) Each group contains 36 root trainer compartments. All the root trainers in each group were inoculated with the same* G. pallida *population in order to avoid any cross-contamination. A single sprouted bud from a specific potato genotype was placed in each root trainer in a complete randomised design. (b) The root trainers allow the development of roots in parallel. (c) Seven weeks after inoculation, the number of females feeding on roots were counted and collected. The females are visible as small white spheres developed along roots (scale bar equals 1cm).*

In each experiment, a Newton and Farcet field (standard) population along with their 4 selected sub-populations were tested on 9 potato genotypes (lines) in total. However, one line (Arsenal) was used in the Farcet screening tests only once, due to the lack of availability of tubers. Seven weeks post-inoculation, the root trainers were opened carefully without disrupting the developed root system, and were placed under a magnifier lens. Females that were visible on the root surfaces were counted and collected using fine tweezers without damaging them and stored in

sterile Eppendorf tubes. The collected nematode material was frozen in liquid nitrogen and subsequently stored at -20oC for later use.

The number of females was calculated as a mean number of the counted developed females for each 'population x potato line' interaction. The mean number from both technical repeats per population was calculated. Prior to this, paired t-tests (p-value ≤ 0.05) were carried out in order to find whether each specific 'population x potato line' application differed significantly in each technical repeat. In general, in both experiments each 'population x potato line' showed a similar trend with regard to a number of developed females. Statistical analysis was performed using the software SigmaPlot Version 13.0 (Systat Software Inc., San Jose California USA, www.systatsoftware.com).

All of the Newton sub-populations had very high reproductive ability on the susceptible Désirée control, varying from 84 to 150 developed females 7wpi (weeks post infections). Royal showed average levels of susceptibility to most of the selected populations whereas Arsenal and Innovator displayed very low susceptibility (i.e. less than 8 females at 7wpi). The sub-populations n-8906 and n-11305, which were both selected on *S. vernei*, showed a substantially increased reproduction rates on potato lines containing the *S. vernei* resistance source (Sv_8906 and Sv_11305) when compared to the unselected population on those lines (Figure 3). By contrast, the sub-populations selected on *H3*, (n-11415 and n-12674) displayed very low multiplication rate on the lines containing *S. vernei* resistance.
A similar pattern was observed with the populations selected on *H3*, the lines selected on H3 (n-11415 n-12674) showed high reproduction on the potato lines containing H3 (Sa_11415 and Sa_12674). These sub-populations also showed an increased reproductive ability on the cultivar Vales Everest, which contains H3. Populations selected on *S. vernei* showed no increased reproduction on cultivars containing H3.

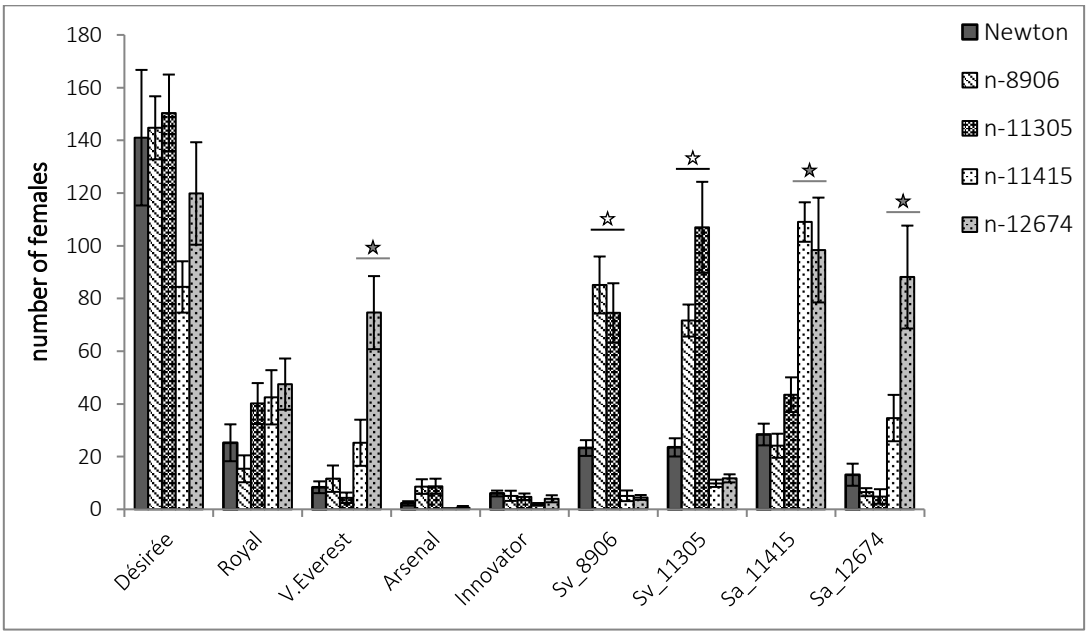

Figure 3: *Number of G. pallida females of the Newton field population and its 4 sub-populations selected on* S. vernei *(n-8906, n-11305) and* S. tuberosum *ssp*. andigena *CPC2802 (H3) (n-11415, n-12674) tested on nine different potato lines. Each bar represents the mean number of developed females from 2 technical repeats each consisting of 4 independent replicates. Désirée is the most susceptible potato line, whereas Arsenal and Innovator the least susceptible. White stars indicate significantly higher reproduction rates, within each potato line, of the nematode populations n-8906 and n-11305 selected on* S. vernei *resistance source when tested on the potato lines containing the same resistance source. Grey-filled stars indicate significantly higher reproduction rates, within each potato line, of the nematode populations n-11415 and n-12674 selected on CPC2802 (H3) resistance source when tested on the potato lines containing the same resistance source. Error bars stand for standard error of the mean (p-value = 0.05).*

Analysis of population Farcet and its selected subpopulations gave a very similar pattern – lines selected on a particular resistance source showed specifically increased virulence against that resistance source (Figure 4). However, one notable exception was that one population (f-11415) selected on H3 also showed increased ability to reproduce on the cultivar Innovator, which contains strong resistance derived from *S. vernei*.

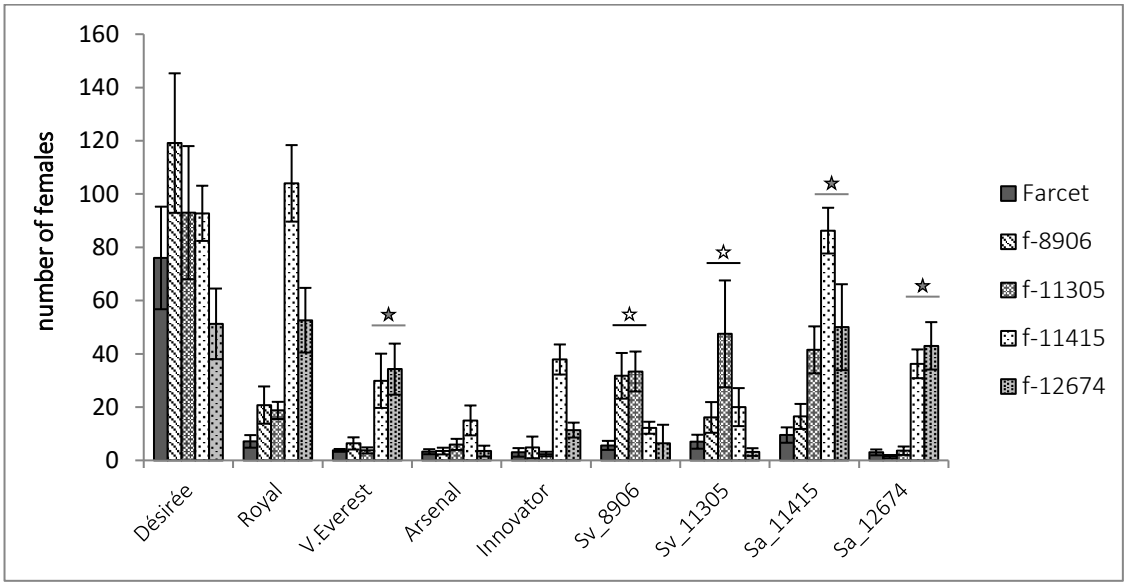

Figure 4: *Number of* G. pallida *females of the Farcet field population and its 4 sub-populations selected on* S. vernei *(f-8906, f-11305) and* S. tuberosum *ssp.* andigena *CPC2802 (H3) (f-11415, f-12674) tested on nine different potato lines. Each bar represents the mean number of developed females from 2 technical repeats with 4 independent replicates in each. Désirée is the most susceptible potato line, whereas Arsenal and Innovator the least susceptible. White stars indicate significantly higher reproduction rates, within each potato line, of the nematode population f-8906 and f-11305 selected on S. vernei resistance source when tested on the potato lines containing the same resistance source. Grey-filled stars indicate significantly higher reproduction rates, within each potato line, of the nematode population f-11415 and f-12674 selected on CPC2802 (H3) resistance source when tested on the potato lines containing the same resistance source. Error bars stand for standard error of the mean (p-value = 0.05).*

In summary, these data show that it is possible to select for increased virulence against a resistance source and that this selection is specific rather than representing a general increase in aggressiveness or reproductive capacity.

2. Identification of candidate avirulence genes using capture enrichment sequencing

2a – a new genome assembly for G. pallida

The genome sequence of *G. pallida* was published in 2014 (Cotton *et al.,* 2014). Although this represented a major advance in PCN biology, there are a number of problems with this assembly that meant that it was inappropriate for use in this project. First, the sequence was generated from the population “Lindley”. Our work used different starting populations (Newton and Farcet); previous experience in this area suggested that sequence differences between Lindley and these populations may be larger than the differences between the Newton or Farcet starting populations and the selected lines generated from these populations. In effect, this would make separating the signal from the noise problematic. In addition, the published *G. pallida* sequence is extremely fragmented. Recent developments in long-read sequencing

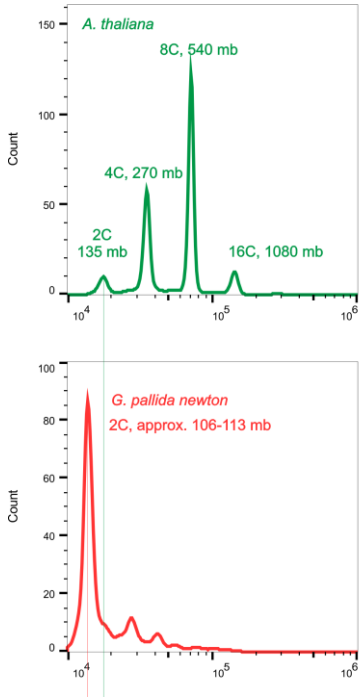

Figure 5: Flow cytometry shows that the genome size of *G. pallida* is between 106 and 113 Mbp. Upper panel shows measurement of the genome of *A. thaliana* for comparison.

technology, most notably PacBio sequencing, offered the opportunity to generate a new assembly for *G. pallida* using one of the starting populations in this study (Newton) at relatively low cost.

In order to guide subsequent bioinformatics work, the genome size of *G. pallida* was first measured using flow cytometry in collaboration with Dr S. Eves-van den Akker at the Cambridge University. This analysis showed that the genome size of *G. pallida* is between 106 and 113 Mbp (Figure 5).

High molecular weight DNA was subsequently extracted from Newton females grown on Desiree using phenol-chloroform extraction, as preliminary analysis showed that commercially available kits produced DNA of smaller fragment size. This DNA was then checked for size and concentration and sent to a sequence provider in Oslo. Four separate DNA PacBio SMRT cells were then used to generate long sequence reads. A total of 34 Gb of sequence was generated with an average read length of 12,575 bases. This sequence was then quality controlled, assembled and polished to generate a new genome assembly for the Newton population. A comparison of the new assembly metrics with those from the published *G. pallida* genome sequence (Table 1) show that the new assembly is considerably less fragmented than the published sequence, with 50% of the sequence residing in contigs in excess of 1,194,397 bp in length.

	Published genome	**New assembly**
Assembly Size (Mbp)	124.6	120.5
Scaffolds	6,873	267
Scaffold N50	121,687	1,194,397
Longest Scaffold	600,076	6,176,216
GC content (%)	37	36
Span of Ns	21,024,229	662,155
BUSCO score	74	89
Number Of Genes	16,000	19,000

Table 1: Comparison of metrics for the published and new *G. pallida* genome assemblies

All of these metrics show that the new assembly represents a considerable improvement on the previously published version. We are currently in the process of completing gene calls and mapping RNAseq reads to this genome assembly. Once complete we will submit this new assembly for publication. This will represent a significant outcome of enormous value to the PCN research community.

2b Identification of candidate avirulence genes

The patterns of selection for virulence in our screening assays were the same for the Farcet and Newton selected lines. We therefore focused our analysis of variation in sequences on the Newton lines in order to be able to take advantage of the new genome assembly that we had generated.
Differences in virulence in plant pathogens are nearly always due to differences in effectors, as it is effectors that are recognised by the products of resistance genes. Our previous work has allowed us to identify the full effector complement of PCN (above). In theory, variation in effector

sequences (polymorphisms or presence/absence differences) can be analysed by resequencing the full genomes of each of the populations being studied. However, sequencing of entire genomes from different populations is laborious and not cost-effective. Effectors make up only a small fraction of the entire genome sequence and sequence depth in other regions is effectively wasted. Target enrichment sequencing (Mamanova et al., 2010) offers a cost-effective and high-throughput approach to identify and study sequence polymorphisms. This method focuses on selectively capturing, enriching and sequencing targeted genomic regions. In plants, it has been developed and applied recently for capturing *R* genes (i.e. *R* gene enrichment sequencing or RenSeq) (Chen et al., 2018; Jupe et al., 2014; Jupe et al., 2013). In this technique, unique biotinylated RNA-based oligonucleotide probes (or baits) of 120nt are designed to bind to complementary previously characterised or potential target genes. Each bait is 50% overlapped (i.e. in 60nt-step) with its neighbouring bait in a 2x coverage (tilling). The gDNA samples are first fragmented into small fragments of a specific size prior the development of DNA libraries. Enrichment is performed through the hybridisation of the PCR-amplified barcoded DNA library with the biotinylated baits (Figure ; step ii). The baits are subsequently captured using streptavidin-coated magnetic beads (Figure ; step iii). Any non-targeted DNA fragments are washed away and discarded (Figure ; step iv), the magnetic beads are washed away from the captured library which in turn is amplified and sequenced (Figure ; step v).

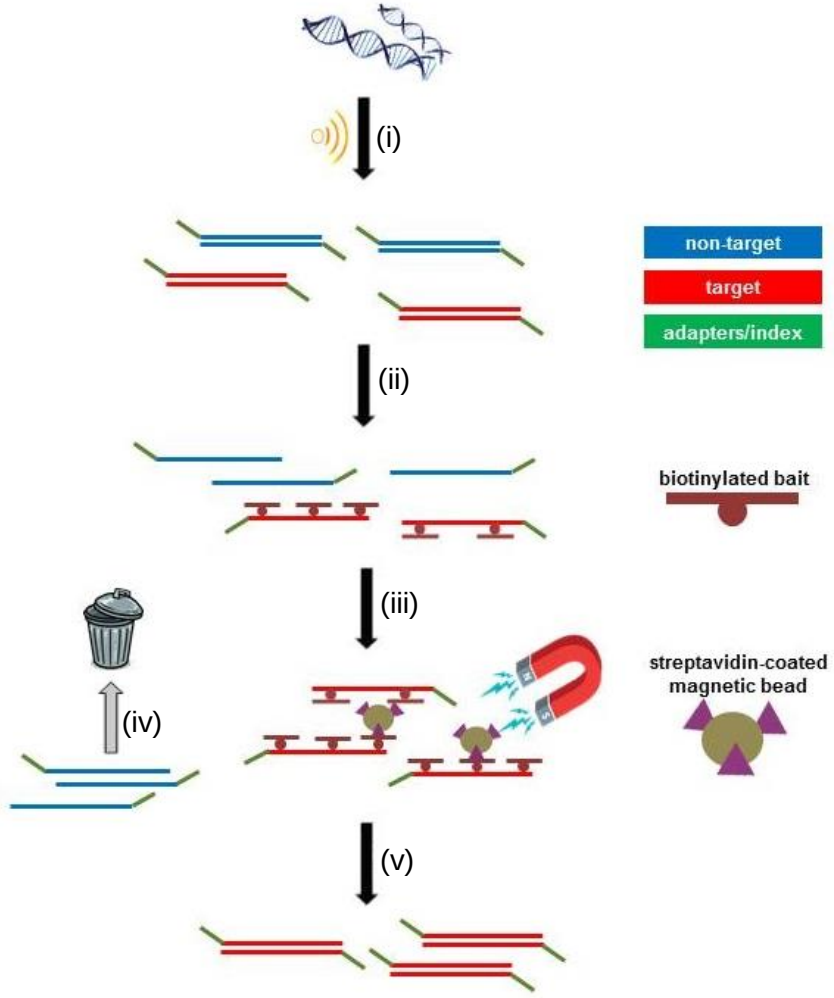

Figure 6: *The workflow of the target enrichment sequencing.*

In this work area we aimed to identify candidate *Avr* genes from *G. pallida* Newton selected for increased virulence on two resistance sources (*S. vernei* and *H3*). DNA libraries from two Newton sub-populations selected on *S. vernei* and two selected on *H3* were constructed and genomic effector-encoding regions were captured and enriched. The captured regions were then sequenced on the short-read Illumina MiSeq platform and the diversity of the sequences from the different populations were studied for candidate *Avr* genes identification.

Probe design and target enrichment sequencing
In total, 24,744 biotinylated RNA-based oligonucleotides were designed *in silico* to capture genes that encode *G. pallida* effectors. About 611 out of 700 total targets were fully covered by the designed baits (Table 2).

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# of total baits	Predicted number of targets with x% bait coverage (Total target genes = 700)									
	100%	**90%**	**80%**	**70%**	**60%**	**50%**	**40%**	**30%**	**20%**	**10%**
24,744	611	647	667	683	689	694	695	700	700	700

Table 2: *Predicted number of target-genes with a corresponding bait coverage (%) of the designed baits. From the total 700 targets, 611 were fully covered by the designed baits*

The target-enriched DNA from the 5 *G. pallida* selected sub-populations were then sequenced on a single cell of Illumina MiSeq platform using 2 x 250bp paired end reads. In total 23,590,596 reads were obtained and over 97% of these were assigned to one of the 10 indexed libraries. All libraries were represented approximately equally in the sequencing data (Figure).

2.1.1.

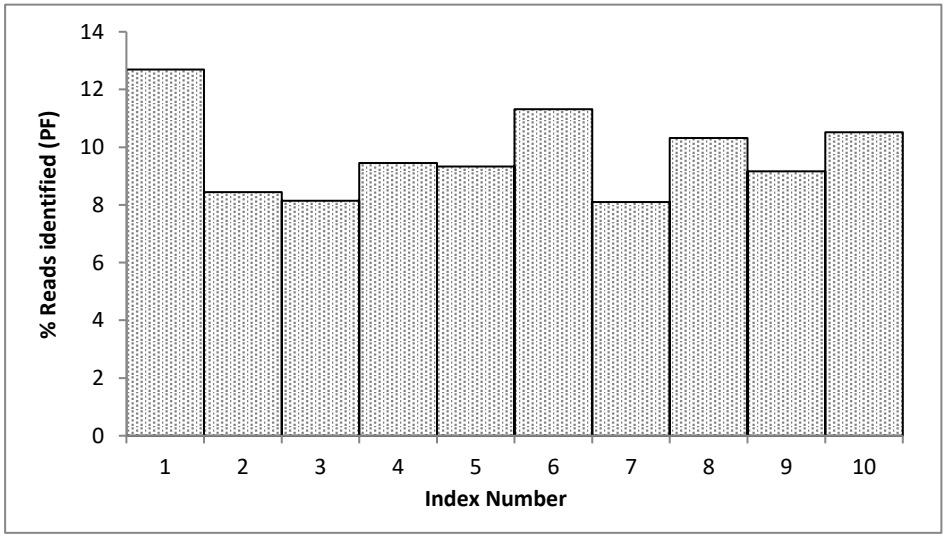

Figure 7: *Overview of the sequence depth of the 10 enriched libraries. PF stands for the total fraction of the reads assigned to a specific index.*

Diagnostic real-time PCR of the captured enriched libraries
In order to test whether the capture process had resulted in enrichment of DNA sequences encoding effectors, a real-time PCR (qPCR) was performed using primer sets that amplify known effectors (SPRY-414-2, SPRY-1719-1 and G16H02) of *G. pallida*. The non-effector gene *GAPDH* was used as endogenous control. Analysis of these data (Figure 8) showed a highly significant increase (>100 fold) in the relative expression of all the genes in the post-enrichment library. This analysis confirms that the enrichment procedure had worked as planned, with effector encoding sequences being present at much higher concentration in the libraries after enrichment.

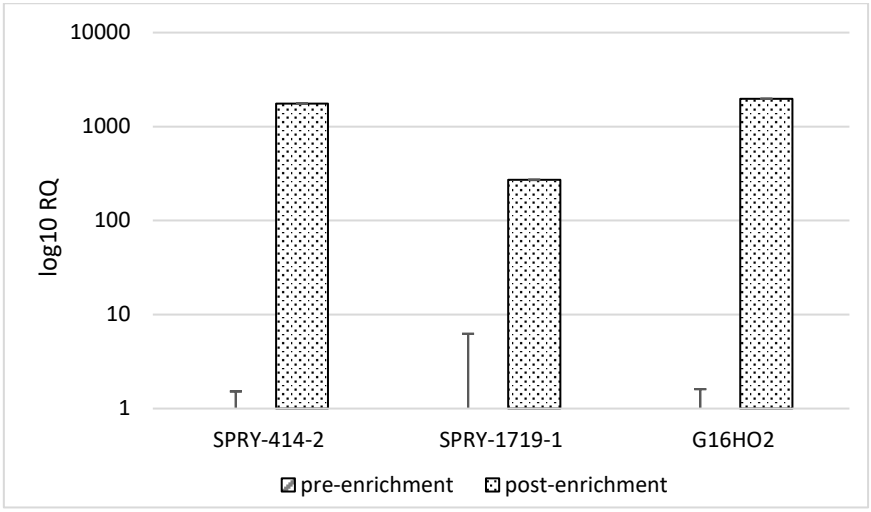

Figure 8: *Comparative relative quantification (RQ) of the pre- and post-enrichment library of the three effector genes (SPRY-414-2, SPRY-1719-1 and G16H02). The non-effector gene GAPDH was used as endogenous control. Error bars stand for 95% confidence interval (CI). RQ values are presented in a logarithmic scale log10.*

The bioinformatic pipeline used for analysing differences in the sequences derived from the starting and selected lines is summarised in Figure 9. Reads were first aligned to the new genome assembly and polymorphisms were identified. This gives rise to a very large number of potential SNPs which were filtered in order to focus on those in coding regions that give rise to a change in amino acid sequence of the protein encoded by that gene. Finally, the polymorphisms present in genes represented on the capture array sequence were identified.

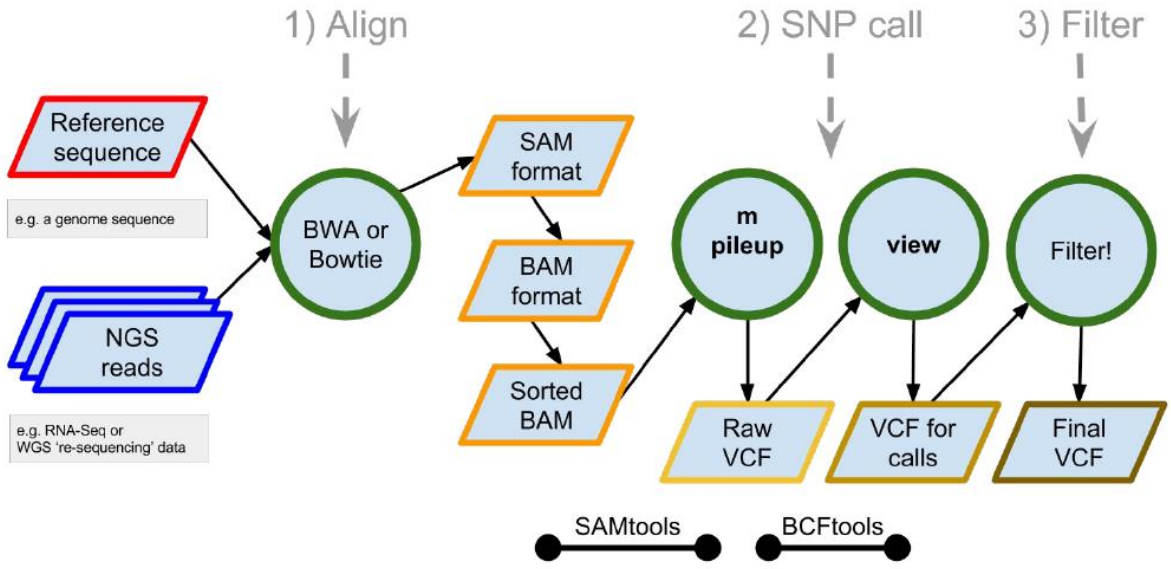

Figure 9: Pipeline for bioinformatic analysis of polymorphisms in selected lines of *G. pallida*

To date polymorphisms have been identified in 55 potential effector sequences. Current work is aimed at further narrowing this figure by comparing the lists of genes from selection on the different potato lines containing the same resistance source. These identified genes represent candidate avirulence genes recognised by H3 and *S. vernei* resistance sources. Future work in this area will include functional testing of the candidate genes (below) to confirm which are recognised by the resistance source. Once the recognised effector is identified we will analyse variation in these effectors across UK PCN populations in order to determine likely durability of the resistance source. It may also be possible in the longer term to develop a diagnostic tool

3. Development and testing of a system for functional testing of candidate avirulence genes

Note: This area of work was codeveloped with another PhD student, Shona Strachan, working on a project co-funded by AHDB

The analysis described above led to the identification of candidate Avr genes. These data were generated in the later stages of this project, meaning that it has not yet been possible to test functionally which of the sequences represent the genuine Avr genes for H3 and *S. vernei* sources. During this project we optimised a system that allows functional testing of Avr genes using candidate Avr genes recognised by H1.

As a part of the *G. rostochiensis* genome project, populations of all five *G. rostochiensis* pathotypes were re-sequenced and mapped against the reference genome assembly (generated from pathotype Ro1). In total 190 genes had potential for modified or loss of function between the populations that are avirulent and virulent against H1. When these 190 genes were then cross-referenced with the effector list for *G. rostochiensis*, only two encoded proteins similar to those on the effector list. These two genes (GROS_*g13394* and GROS_*g12477*) were therefore characterised as candidate *Avr* genes (Eves-van den Akker et al., 2016). The gene GROS_*g13394* encodes a putative cellulose binding protein produced in the subventral gland cells and its sequence is similar to *GLAND10*, which is expressed in the esophageal glands of the pre-parasitic *H. glycines* J2s; GROS_*g12477* encodes an ubiquitin-like protein produced in the dorsal gland cells and is similar to a protein found in the parasitic *G. rostochiensis* J2s involved in suppression of immune responses (Chronis et al., 2013; Eves-van den Akker et al., 2016; Noon et al., 2015). Our initial analysis showed that one of these sequences (GROS_*g12477)* was a poorly predicted gene. All further analysis was therefore focused on the GROS_g13394 gene.

We first cloned this gene from cDNA generated from virulent (Ro5) and avirulent (Ro1) nematodes. This analysis confirmed that sequence polymorphisms exist in this gene from the two pathotypes (Figure 10).

```
                          10         20         30         40
                   ....|....| ....|....| ....|....| ....|....|
conseq_Ro1_g13394sp  1   MNGLIGILSF GFFICATVVL AQPSTSPVIV SVELVNSTEN  40
conseq_Ro5_g13394sp  1   .......... .......... .......... ..........  40

                          50         60         70         80
                   ....|....| ....|....| ....|....| ....|....|
conseq_Ro1_g13394sp  41  HYNYSLEFTG NIFKLICQVT FQVQLPDGAI LEKYWNMNPV  80
conseq_Ro5_g13394sp  41  .......... .......HL. .....QN... ..........  80

                          90         100        110        120
                   ....|....| ....|....| ....|....| ....|....|
conseq_Ro1_g13394sp  81  NGTDNKQFTL PDNVRLYPGQ SFADAGITVA GGGGEPEVTI  120
conseq_Ro5_g13394sp  81  .......... .......... .......... ..........  120

                          130
                   ....|....| ....|...
conseq_Ro1_g13394sp  121 VAMXSVLSTK KCPDSAG* 138
conseq_Ro5_g13394sp  121 ...K...... .......* 138
```

Figure 10: *Comparison of g13394 sequences from Ro1 and Ro5 confirms differences in sequence between these populations*

We then transferred the cloned sequences into a Gateway compatible PVX viral vector pGR106GW. This vector allows expression of viral RNA from *Agrobacterium* following infiltration into plants, thus allowing expression of the protein cloned into the vector. Two potato varieties were used; Désirée (as a susceptible control variety) and Maris Piper (which contains *H1*). Following initial tests of a range of methods for expression of the proteins in potato, vacuum infiltration of potato leaves was found to be the most efficient means of expressing proteins in leaves. For this, potato leaves were placed into a beaker containing culture of *Agrobacterium* containing the appropriate genes and exposed to a vacuum. This allowed

culture medium to enter into air spaces within the leaves. Potato leaves were subsequently left for several days in a moist environment to allow symptoms to develop. As controls we also included constructs that should give rise to no response (GFP) and to a strong cell death response (CRN2).

Desiree responded strongly to all constructs that were applied (Figure 11). This may indicate a response to the strain of *Agrobacterium* that was used for these experiments and will require further optimisation in future. Results with Maris Piper were more encouraging. A much greater cell death response was seen in response to the CRN2 construct when compared to GFP, and this difference was statistically significant. However, Maris Piper showed a slightly (although not significant) stronger response to the GROS_g13394 gene from Ro5 compared to that from Ro1, which is not what would be expected for Avr H1. We can therefore conclude that GROS_g13394 gene is unlikely to be the effector recognised by H1. However, this system clearly offers a route for expression of candidate Avr genes in potato.

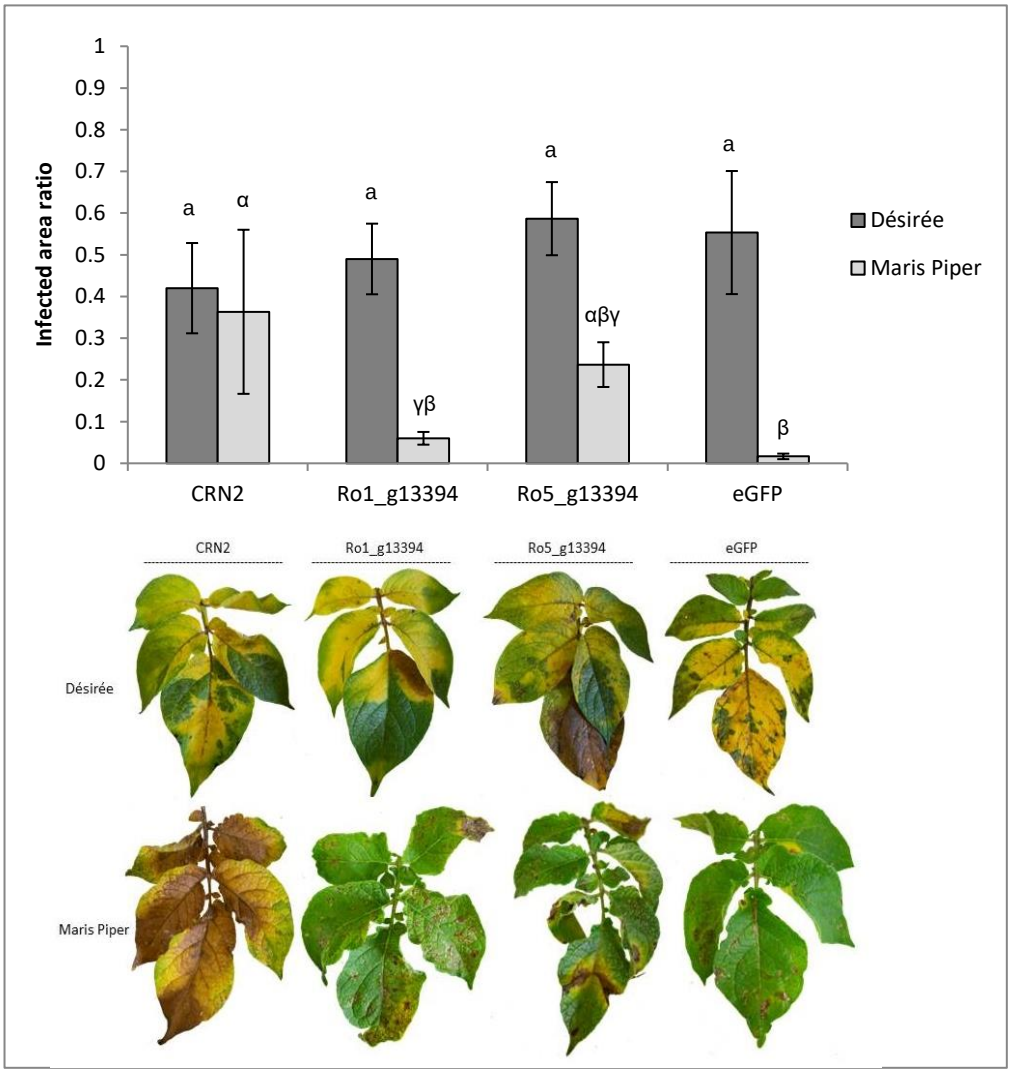

Figure 11: *Vacuum infiltrations of Avr H1 candidates and controls. In the barchart, each bar represents the ratio of the necrotic area to the total leaf area. Dark grey bars show the infected area by each construct on Désirée leaves and the light grey bars on Maris Piper. eGFP and CRN2 were used as a non-recognised, negative control and positive control respectively. Error bars stand for standard error of the means. Latin characters indicate statistical differences within Désirée and Greek characters within Maris Piper determined with a two-way ANOVA test (Duncan's method, p-value < 0.05, n = 4). The photos show indicative examples of the inoculated leaves for each construct.*

Conclusions and Recommendations

The highly variable and complex genetic make-up of UK *G. pallida* populations means that there is a pool of genetic variation present from which selection of virulent nematodes can occur. In keeping with this, a population of *G. pallida* has recently been reported from continental Europe which is no longer controlled by Innovator. **Resistance still represents the best way to control *G. pallida*** and growers should be encouraged to use this wherever practically feasible. In the longer term, **combining a range of different resistance sources in a single cultivar will provide more durable resistance that will be extremely difficult for PCN to overcome** and we would recommend that this forms a focus for future projects for AHDB.

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4. KNOWLEDGE TRANSFER ACTIVITIES

- European Society of Nematologists, Braga (September '16): poster **(Identification of Avr genes in *Globodera pallida* as tools for assessing durability of resistance**)
- AHDB studentship conference (November '16): poster (**Identification of Avr genes in *Globodera pallida* as tools for assessing durability of resistance**)
- Cellular & Molecular Sciences JHI seminar (March '17): talk (**Identification of Avr genes in potato cyst nematodes and assessment of durability of resistance)**
- Molecular Biology of Plant Pathogens (BSPP), Durham (March '17): poster (**Identification of Avr genes in potato cyst nematodes)**
- SPIT nematode effectors meeting, St Andrews (September '17): talk (**Identification of Avr genes in potato cyst nematodes)**
- AHDB studentship conference (November '17): talk **("Catch me if you can": Identifying Avr genes in potato cyst nematodes)**
- AAB Advances in Nematology conference, London (December '17): talk **("Catch me if you can": Identifying Avr genes in potato cyst nematodes)**
- Uni of St Andrews-Biology school PG conference (January '18): talk **("Catch me if you can": Identifying Avr genes in potato cyst nematodes)**
- European Society of Nematologists, Ghent (September '18): talk **(Identification of Avr genes in *G. pallida* through target enrichment sequencing – A new insight into the *G. pallida* genome)**
- Presentation to US *G. pallida* consortium (GLOBAL), Cornell University. (March 2018). **Capture enrichment of effector encoding sequences for identification of avirulence genes**

5. ACKNOWLEDGEMENTS

Dr Vivian Blok Co-supervised this project.