Final Report

11120017 Fellowship: Development of a Succession Potato Nematologist

Reporting Period: October 2015 – October 2018

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Report No. 2019/6

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1. SUMMARY

1.1. Aim

The purpose of the nematology fellowship was to employ a fellow for 3 years to work with nematologists at SASA to develop a thorough understanding of the field and the skills necessary to continue as a proficient nematologist in their own right.

1.2. Methodology

A range of milestones relating to nematode identification skills and management were agreed. The fellow worked through these and new objectives were introduced as and when required. These included providing training and proficiency testing for GB laboratories in the detection of and identification of Potato Cyst Nematodes (PCN). The fellow was also involved in research to establish the most appropriate soil sampling rates for detection of PCN, had input in to a guide for the management of PCN and carried out a project with a student to determine growers attitudes to PCN management.

1.3. Key findings

The fellow was involved in a project which determined appropriate soil sampling protocols for detection and quantification of PCN. The conclusions of the work are provided in a separate report “PCN Soil Sampling”. This was the basis for the development of standardised soil sampling protocols in land destined for ware production. The protocols are summarised in the pdf “PCN – Sampling and Laboratory Guide”

As a result of the fellowship the quality of detection and identification of PCN across laboratories in GB has improved following on from the training provided. The fellow has assessed current PCN management practices in GB and determined that more action is required to sustainably manage PCN for the future. As the fellowship was based at SASA there has been a focus on PCN management in Scotland. The fellow developed a profile as a nematologist within the EU and international nematology community and has provided advice at an international level. She attended and presented at several nematology and potato meetings in order to disseminate many of the findings of the work.

1.4. Practical recommendations

Following on from the project the fellow recommends a greater level of knowledge exchange between science and growers. Land should be tested to determine PCN presence and quantity. Laboratories should continue with a programme of self-assessment/proficiency testing. Potato breeding should concentrate on incorporating *G. pallida* resistance in cultivars for both the table market and processing to allow better tools to manage PCN. Effective useable management options should be developed for cooler climates.

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2. INTRODUCTION

There are only a small number of nematologists currently working in GB and those specialising in plant parasitic nematodes are particularly rare. The aim in training a succession nematologist was to impart key skills from three specialist nematologists who currently work at SASA. The nematology laboratory manager has over twenty years of morphological diagnostic experience in both cyst nematodes and in free living nematodes. She is also responsible for managing the day-to-day statutory work and therefore has an excellent understanding of the entire process. The molecular ecologist who developed the current PCN real time PCR diagnostic system used at SASA, will often create new assays and has many years' experience in molecular techniques and problem solving. The section head of Virology and Zoology at SASA, studied nematology at post graduate level and brings a lifetimes wealth of experience in the management and control of PCN. He is regularly asked for input and advice on a global scale about this subject and he helped to develop the current EU regulations for PCN. This report pulls together three distinct areas that the fellow was involved in working in: soil sampling, providing training to and proficiency testing of commercial laboratories in GB and also an assessment of growers' attitudes and the application of PCN management in Scotland.

2.1. Background on PCN

Potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are considered to be one of the greatest concerns for economic potato production in Britain. Economic losses from this pest are estimated at approximately £50M per annum approximately £10M is spent each year in controlling the pest through the use of nematicides (http://randd.defra.gov.uk/Default.aspx?Module=More&Location=None&ProjectID=9913). PCN feed on the roots of potato plants, compete directly with the plant for resources and limit root growth. For this reason plants are often stunted and low yielding, particularly when plants are facing additional stresses. Severe infestations of PCN can lead to plant death. Potato cyst nematodes *G. pallida* and *G. rostochiensis* were introduced into Europe from South America during the 19th century and since then have spread to most potato growing regions worldwide, and it is believed there were several introductions during this time (Plantard *et al.*, 2008)

2.2. Current PCN situation in Scotland

In Scotland approximately 27,500ha of potatoes are grown annually of which 12,700ha are seed potatoes, the estimated value of the crop in 2016 was £209M https://www.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/agritopics/Pots). Since 1973 when the UK entered the EU, all land intended for seed potato production has required a clear soil test to indicate freedom from PCN. Prior to 2010 SASA tested land at a rate of 600ml per 4 ha, this was one of the lowest rates in Europe. In 2007, an EU directive was agreed to standardise practices across Europe. This implemented a standard rate of 1500ml of soil per hectare, although this could be reduced to 400ml per hectare where previous freedom from PCN has been demonstrated or where rotations are greater than 1 in 6. Most of the seed land in Scotland falls in to the 400ml per ha category (further reductions to 200ml can be made in large fields). The greater the quantity of soil tested the more likely PCN is to be found, so from 2010 onwards there was a significant increase in the amount of land being taken out of seed production. Where PCN are found the land is recorded as being infested with PCN and no seed potatoes may be grown. Land remains recorded unless a subsequent sample tests clear

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from PCN in which case the land is de-recorded. Figure 1 below shows the cumulative data from land recorded with PCN from 1973 until 2017.

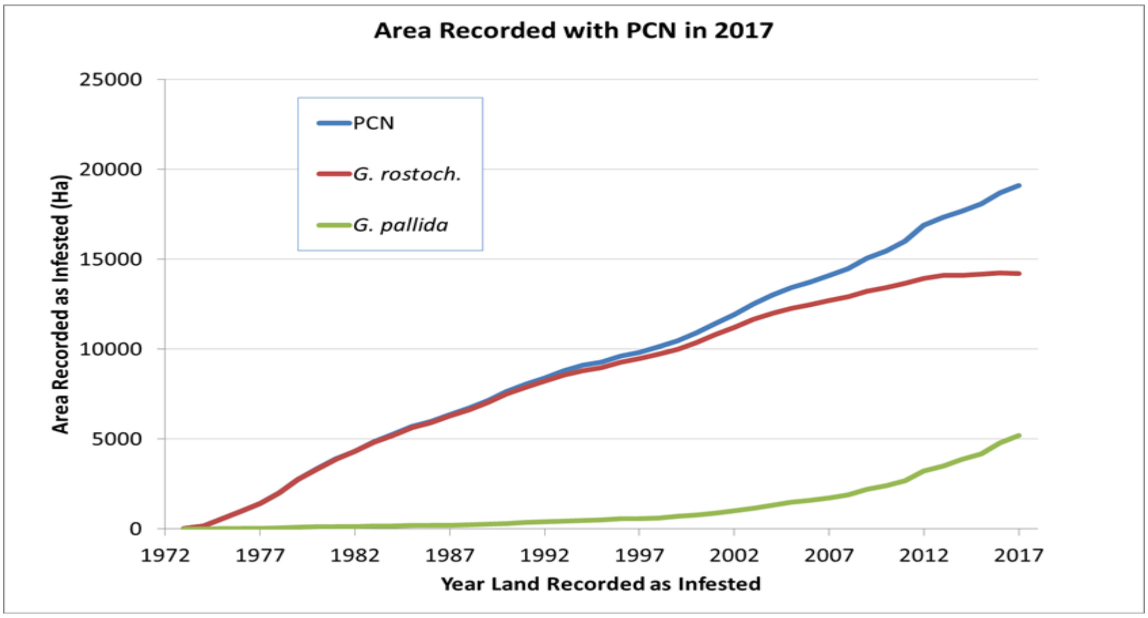

Figure 1: *cumulative area of land recorded with PCN since 1973*

The area of land officially recorded as infested with PCN in July 2017 was 19,108 ha. Of this total, 14,217 ha (74%) was recorded as infested with *G. rostochiensis* and 5,214 ha with *G. pallida* (27%), including 322 ha (1.7%) with both species. Until 1990 almost all infestations were with *G. rostochiensis*, however *G. pallida* infestations have been increasing rapidly since 2000, in 2017 3.1% of land was recorded with *G. pallida* and 2.2% with *G. rostochiensis*.

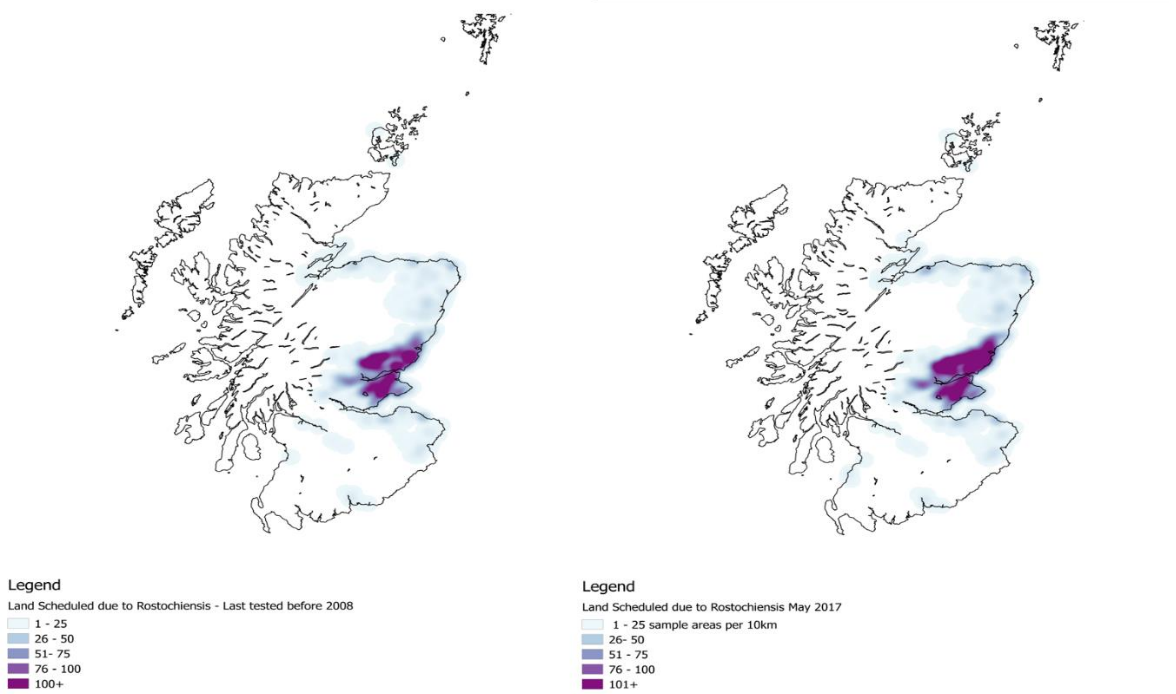

Figure 2a: *Land in Scotland recorded with* G. rostochiensis *in 2007 and 2017*

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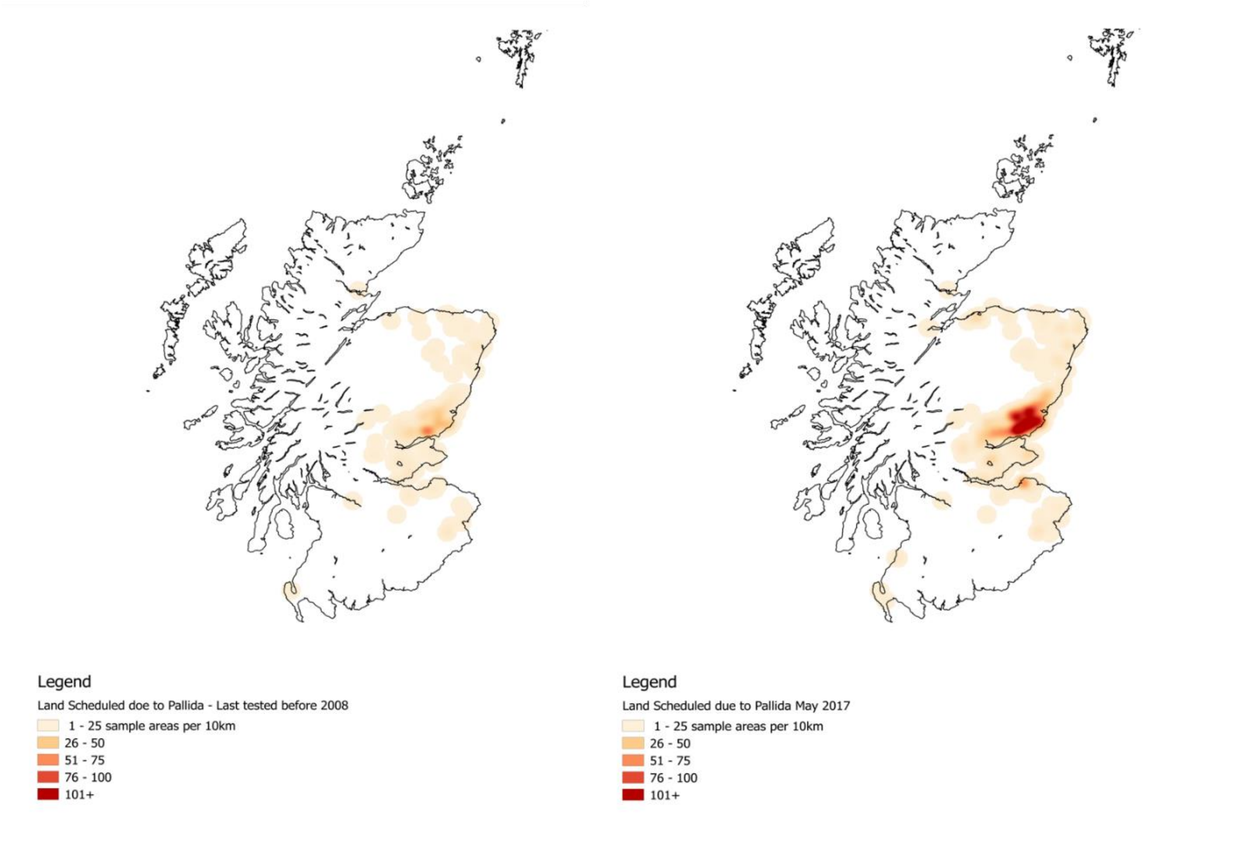

Figure 2b: *Land in Scotland recorded with* G. pallida *in 2007 and 2017*

Figure 2a and 2b show heat maps of PCN infestations by species, demonstrating how PCN infestations have developed over a 10 year period in Scotland.

Table 1*: Land recorded with PCN in Scotland in 2010 and 2017*

G. rostoch. in 2010 (Ha)	*G. rostoch.* in 2017 (Ha)	*G. rostoch.* increase	*G. pallida* in 2010 (Ha)	*G. pallida* in 2017 (Ha)	*G. pallida* increase
13,453	14,217	6%	2,411	5,214	116%

While the total amount of land recorded in 2010 with *G. rostochiensis* was far greater than *G. pallida* with 13,453 and 2,411 respectively, the increase in the land recorded with *G. pallida* was far greater and 116% compared to 6% with *G. rostochiensis* over a 7 year period. This data suggests that while *G. rostochiensis* populations have broadly levelled off, *G. pallida* populations are continuing to increase. While more land than ever is now being recorded with *G. pallida*, it seems that once infested it is more difficult to de-record this land than is with *G. rostochiensis.* Over a seven year period, new infestations of *G. rostochiensis* have been found on an average of 345 ha each year, whilst an average of 233 ha have been de-recorded, resulting in a nett increase in *G. rostochiensis* infested land of 112 ha p.a. Over the same period, new infestations of *G. pallida* have been found on an average of 448 ha each year, whilst an average of just 48 ha have been de-recorded, resulting in a nett increase in *G. pallida* infested land of 400 ha p.a. (Pickup *et al*, 2018).

An annual ware survey is also carried out under the EU directive (2007/33/EC) which requires 0.5% to be tested annually, approximately 80ha per annum. Over the six year period from 2010-2016, 66% of land tested was found to be clear of PCN, 26% had *G. pallida* and 13% had *G. rostochiensis* (Figure 3).

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County	Total area tested (Ha)	PCN incidence in ware land	PCN incidence in seed land
ANGUS	190.1	40%	10.2%
FIFE	110.2	36%	5.9%
PERTH	64	31%	4.6%
EAST LOTHIAN	48	58%	2.1%
ABERDEEN	42.9	28%	1.6%
Others	108.5	15%	1.7%
Grand Total	563.7	34%	5.4%

EU 2007 Directive requirement to survey 0.5% of ware land p.a.

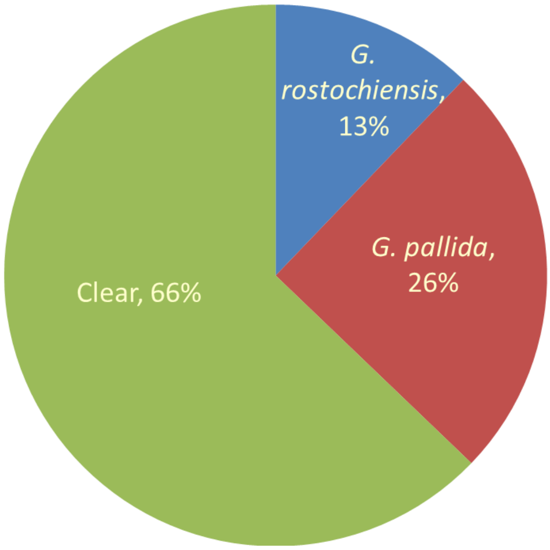

Figure 3: *Pie chart showing outcomes of ware survey in Scotland from 2010 – 2016, table detailing land recorded as infested by region and whether seed or ware*

Overall there has been a marked increase in the amount of land that has been taken out of seed production in Scotland due to the presence of PCN since 2010 when the sampling rates changed. However, since 2011 the land infested with *G. pallida* has increased by 94% where over the same time period *G. rostochiensis* only increased by 4% demonstrating a strong shift towards *G. pallida*. If this trajectory continues, where the amount of land taken out of production due to *G. pallida* continues to double every 5-6 years there will come a point where it is no longer feasible to produce seed potatoes in Scotland unless something changes, and this may happen as soon as 30 years from now.

One of the most important factors in determining both the distribution of PCN in any land and also how effective any management options have been in tackling the problem is soil sampling.

2.3. Sampling for PCN

With every susceptible potato crop that is grown, PCN can multiply up to 100-fold (Evans and Kerry, 2007) and will, if not appropriately managed, lead to land becoming unsuitable for the commercial production of potatoes. To determine whether PCN are present in land and, if present, to assess the species and population level, field soils are sampled and tested for PCN. In a one hectare field, the top 20 cm depth of soil equates to 2 million litres of soil, only a very small proportion of which can feasibly be tested. PCN are unevenly distributed on a horizontal scale across the field. This aggregated distribution reduces the probability of detection. Therefore, the challenge for soil sampling is how to take a soil sample that best represents the PCN status of the field for submission for laboratory testing without the cost becoming prohibitive. The larger the sample, the more expensive the test will be. Field sampling costs are mostly affected by the time taken to draw the sample, this will depend on how many cores and the pattern used to sample the field.

Finding low level and patchy PCN infestations in a field is particularly challenging. A single cyst in a 400 ml sample of soil drawn from 1 ha roughly equates to a population of PCN of 5 million cysts/ha, a level which is likely to have taken several field generations (typically at least 4, depending on rotation periods and varietal

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susceptibility) to reach a detectable level. Earlier detection of PCN would require soil sampling at a rate that is at least an order of magnitude more intensive and with a proportionate increase in laboratory costs. Therefore, it is unrealistic to expect to be able to reliably detect PCN populations at very early stages of infestation. Fortunately, detection of PCN by soil sampling is feasible before the pest can cause significant economic damage. Once present in fields, the tools to manage PCN and provide commercial yields of potatoes are available. Monitoring and estimating population levels of PCN is critically important in informing the management decisions required to sustainably manage PCN populations in infested land and to minimize the economic impact of PCN on the potato crops grown in that land.

Therefore, there are two purposes for testing fields for PCN: detection i.e. is PCN present; and quantification, i.e. how much PCN is present. Testing fields for PCN involves two key elements: soil sampling and the subsequent laboratory analysis.

In terms of PCN testing prior to seed potato production, the sampling criteria are already laid out by the European Council PCN Directive (2007/33/EC) as discussed above. Both species of PCN are listed by EPPO on the A2 list, i.e. quarantine pests that are locally present in the EPPO region. Directive 2007/33/EC recognises that although a quarantine organism, PCN is present in a significant proportion of the ware potato production area of the EU and therefore it would be unreasonable, given the measures available to manage PCN, to prohibit potato production on infested land. There is a requirement to monitor PCN populations in ware potato land to provide the necessary information to underpin management programmes and to evaluate the effectiveness of such programmes.

There has been a lack of clear guidance on the best method to sample fields destined for ware production. Several sampling strategies are deployed across the industry and the relative merits of the different approaches had not been critically assessed. When sampling a field for PCN, the smaller the size of the block or area from which the sample is drawn, the greater will be the discrimination between areas of differing PCN incidence. Consultation with industry indicated that in Britain most growers work on a scale of a 1 ha block and the work developing standardised soil sampling protocols for PCN (Soil Sampling for PCN) was based on sampling units of 1 ha.

2.4. Factors influencing PCN population dynamics

The development of cyst numbers from year to year at a single site depends on a number of factors, including species of PCN, potato cultivar, rotation length grower control interventions, temperature and the initial PCN population (van den Berg *et al*., 2006; Trudgill *et al.,* 2014; Kaczmarek *et al*., 2014; Evans, 2015).

2.5. Development of PCN infestations

PCN can be introduced into a field by a number of means. Introduction by contaminated seed has been traditionally considered as the most likely means, with population build-up around the original introduction site forming a focus within the field. This could occur more than once due to the simultaneous introduction of several sites of infection produced by planting the same infected seed lot, thus leading to the development of several foci within one field. Repeated cultivation of the field will lead to an increase in the spread from the original populations. Subsequent introductions will also lead to the build-up of additional foci.

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Other potential means of introduction are from water courses, either by rivers and streams carrying cysts from infested land and subsequently flooding onto potato land or through use for irrigation, or through run-off from neighbouring infested fields. In these cases the resulting field distribution is likely to be different from those introduced by the planting of infested seed. Strong winds can also be responsible for large-scale movement of PCN, particularly in areas where soils can be dry for prolonged periods. PCN can also be introduced on the feet of animals or with the movement of contaminated machinery. The means of introduction will determine the initial population that will be bulked up by each subsequent susceptible crop.

The population of PCN will increase with every susceptible crop that is planted. Shorter rotations allow the population to increase more rapidly, with potential 50-fold increases occurring on each host crop and shorter intervals between host crops providing less time for natural population decline. Shorter rotations are also more likely to intensify the aggregated distribution of PCN in the field as there is less time for foci to be dispersed around the field during general cultivation of the land. Typical rotations for ware crops in Britain are around five years (Minnis *et al*., 2002). In the Netherlands, where highly aggregated populations of PCN have been recorded, many infestations are on relatively recently reclaimed land so it is likely that the PCN were originally introduced with seed and then increased under frequent rotations. Typically potatoes are grown every three years in the Netherlands, potentially increasing PCN populations to detectable levels in as few as four rotations, and producing the highly aggregated distributions described by Been and Schomaker (2000). Once PCN are so abundant, spread by other means within field becomes more likely – and the PCN distributions are likely to become less heterogeneous.

In Britain, some PCN infestations are known to be 70 or more years old, e.g. SASA has evidence from fields near cities in Scotland suggesting potato crops were grown on almost annual rotations during the Second World War to meet with demand and limited availability of transport. Consequently, populations of PCN reached very high levels, inevitably leading to poor yields. The land has subsequently been brought back into potato production with the PCN population managed by long rotation. In these cases the expectation is of far more homogeneously distributed populations of cysts. Extensive cultivation of the land over many years will lead to a widespread distribution of PCN across the field. In reality there will be many distributions of PCN with levels of aggregation that are intermediate between these two extreme scenarios.

2.6. Threshold values for PCN management

Two basic factors should be considered in relation to PCN Management: the protection of the crop; and the protection of the land.

Protection of the crop: The point at which PCN begin to cause damage in a crop is dependent upon the population level/viability of the cysts, the tolerance of the potato cultivar, the soil type and other environmental factors and therefore threshold values for PCN are difficult to set. Many threshold recommendations are based on ADAS advice originally provided in the 1970s which suggests that where no viable cysts are found the field is safe for potato cultivation, but the land should be sampled again prior to the next crop. Where 1-10 eggs/g are found they considered this a low value, although at above 5 eggs/g they recommended the use of a nematicide. They classed 11-60 eggs/g as a moderate infestation and over 60 eggs/g a high infestation.

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Protection of the land: A single crop of a susceptible cultivar has the capacity to increase the PCN population by over 50-fold, i.e. taking the population from the limits of detection to highly damaging in one cropping season. Therefore, to protect the land, it is highly advisable not to grow cultivars susceptible to the species of PCN present without taking additional measures to mitigate against the increase.

2.7. Identification of PCN

Potato cyst nematodes can be identified using either morphological features or genetic characteristics. At SASA prior to 2010, soil samples were washed using a Fenwick can flotation method. The float material was collected on filter papers and examined, by experienced diagnosticians using microscopes, for the presence of PCN. Where PCN were detected 3 cysts were removed and dissected to release juvenile worms. The morphological characteristics and morphometric measurements of the juveniles were used to determine the species present using a compound microscope. Where more than 3 cysts were present the remainder were sent for PCR to determine the species composition of the remaining cysts. Visual examination is time consuming and requires thorough and rigorous training in morphological diagnostics along with years of experience in detection of cysts from float material. SASA has a handful of diagnosticians fully trained and experienced in the detection of PCN and speciation however increasing this capacity would take several years. This meant that with a more than doubling in the number of soil samples being tested it was not feasible to continue with this method. Therefore to fulfil statutory requirements and minimise costs a new method was developed (Reid *et al.*, 2015). Cysts are now extracted using a MEKU carousel designed for the high throughput extraction of cysts from soil (Pollähne, Wennigsen, Germany).This system is similar to Fenwick cans in that it is based on sieving and floatation but allows for 200 samples rather than 60 samples to be processed per day. Samples are tracked throughout the process using barcodes linked to information retained on the seed potato users database (SPUDS). Once through the carousel samples are dried and scraped in to Eppendorf tubes. Total DNA is extracted from the float material using a modified commercial plant DNA extraction kit. A real-time PCR was developed in house targeting the ITS1 region which can both detect the presence or absence of PCN and using a second assay can determine which species are present within the sample. Most laboratories in GB still use morphological diagnostics and to do this have largely relied on in house training and experience.

Part of the requirements of the fellowship was to become proficient in both the morphological and molecular identification of PCN. When the post holder began the fellowship they were already proficient in the morphological detection and identification of PCN. Since beginning the fellowship they have also completed training in the molecular diagnostics and providing results to growers producing outputs from the real-time PCR.

2.7.1. Proficiency Testing

A proficiency test was set up in 2015 in response to industry concerns raised via the Nematicide Stewardship Programme about the performance of laboratories involved in the identification of PCN in Great Britain. AHDB commissioned a proficiency test to be conducted on their behalf by ADAS. The first aim of this was to test laboratories ability to distinguish between PCN, *Heterodera* and other material which could be mistakenly identified as PCN. The aim was to test a laboratories’ ability to distinguish to species.

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The second aim was to determine the laboratories' ability to extract cysts from soil samples and carry out quantification of PCN populations.

One of the first assignments for the nematology fellow was to participate in this initial proficiency test as it would provide an assessment of their capability in morphological identification and serve as a refresher in an area they had not worked in for a few years.

While a few of the laboratories performed well, the results from the initial proficiency test showed limited ability of some laboratories to identify grass cyst nematode *Punctodera spp*. and *Heterodera spp.* cysts as different from potato cyst nematodes. It was recommended that extra training was provided. A two day training course was provided by SASA in August and September 2016 through the AHDB fellowship programme. Participants from five laboratories in GB were given background information and training in the morphological identification of cyst nematodes present in the GB and management options for PCN.

2.8. Management of PCN

Eradication of PCN is not considered to be possible except in areas where an initial introduction has been discovered early and measures have been taken to both eliminate the nematode and prevent its spread. In GB, PCN are widespread, there are reports of them surviving for as long as 40 years and to depths of 80cm (Been and Schomaker, 2013). Therefore, management to reduce the population, prevent spread and preserve yield should be undertaken by growers where possible.

Sampling for PCN

While it is only a requirement to test seed land for the presence of PCN, in order to effectively manage PCN it is valuable to have an understanding of the infestation within a field. Testing should initially be done for detection, if PCN are found then the species should be determined in order to devise an appropriate management regime for the field. Quantification of the infestation will allow for a better understanding of the scale of the problem and help to determine the most appropriate management options. This will also allow for an assessment of the success whichever management options were chosen.

Use of certified seed

Seed potatoes can only be grown in land that has been tested and found free from PCN. Therefore most management options relate only to ware crops. Ensuring that only certified seed is used from land that has been tested and found to be free from PCN will help to prevent the initial introduction of PCN in to a field. Testing for soil for PCN is not a guarantee that there are no PCN present in the field as the amount of soil sampled will determine the likelihood of detection. However using certified seed means that the land has been tested and the sample has been found free from PCN. While PCN may still be present in the land if it is below detectable levels then the possibility of the spread is limited. This is far safer than using uncertified seed where the land has not been tested.

Control programmes

Where land has been tested for PCN as part of a statutory programme to prevent the production of seed potatoes in infested land, when the land tests positive for either PCN species seed potatoes cannot be produced. Ware however can be produced providing that this is done as part of a control programme. The control programmes in Scotland

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are largely determined using the AHDB PCN calculator for *G. pallida* https://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator. The calculator will determine outcomes given different treatments and recommendations on nematicide use, varietal choice and rotation length will be determined using this. Where *G. rostochiensis* is present generally a resistant variety is recommended as there are many options available for this.

Varietal resistance/species of PCN

Resistant cultivars can help to maintain populations at low levels (Turner and Fleming, 2002). The population of PCN will increase on every susceptible crop that is planted, with potential increases of over 50-fold occurring on each host crop (Evans & Kerry (2007) state a potential increase of 100-fold, but for the purposes of this report we prefer to use a more conservative estimate of 50-fold). Resistant cultivars limit PCN population increases.

Resistance ratings

The 1-9 scoring scale published by EPPO and encapsulated in the EU PCN Directive (EPPO bulletin, 2006) categorizes cultivars according to their resistance, with an increase in the resistance score of 1 point equating to a 50% reduction of the PCN increase (Table 2). Thus if a 50-fold increase from the initial population is recorded on a susceptible cultivar (Score of 2), growing a cultivar with a resistance score of 3 will be expected to result in a 25-fold increase, a score of 4 to result in a 12.5-fold increase, and so on. Highly resistant cultivars with a score of 9 result in a PCN population that is below 1% of final population on a susceptible cultivar and can be expected to produce a reduction in the original PCN population in excess of 50%.

Table 2: Relative susceptibility of cultivars with their relative resistance scores

Relative susceptibility (%)	Score
< 1	9
1.1–3	8
3.1–5	7
5.1–10	6
10.1–15	5
15.1–25	4
25.1–50	3
50.1–100	2
> 100	1

There are a range of cultivars with resistance to both species of PCN, with British growers having access to many cultivars with high levels of resistance to *G. rostochiensis*, but only recently have commercial cultivars with equivalent resistance to *G. pallida* started to become available. Many popular British cultivars, e.g. Maris Piper

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have H1 gene resistance to *G. rostochiensis* (score of 9), but no resistance to *G. pallida* (score of 2), therefore knowing the species of PCN present is essential.

Resistant varieties are highly effective in reducing the PCN population in a field and are potentially the main reason behind the plateauing of land recorded with *G. rostochiensis*. Table 3 shows the most commonly grown cultivars in Scotland for seed and ware potatoes and their respective resistance scores.

Table 3: *Top 10 varieties of seed and ware grown in Scotland and their PCN resistance ratings*

Seed

Variety	Rost.	Pallida
Hermes	2	2
Maris piper	9	2
Cara	9	2
Desiree	2	2
Maris peer	2	2
Atlantic	9	2
Markies	9	2
Pentland Dell	2	2
Charlotte	2	2
Lady Rosetta	9	2

Ware

Variety	Rost.	Pallida
Maris Piper	9	2
Cultra	9	2
Osprey	2	3
Rooster	2	2
Maris Peer	2	2
Saxon	9	2
Harmony	4	4
King Edward	2	2
Vivaldi	2	2
Charlotte	2	2

Information on resistance ratings can be found on the Potato Variety Database

In 2016, 46% of the Scottish seed potato crop had a high level of resistance (score 7-9) to *G. rostochiensis*, whereas only 3% exhibited the equivalent resistance to G. *pallida.* The most popular variety for seed potatoes is Hermes which is susceptible to both PCN species and represents approximately 15% of the total area of seed planted. Hermes is susceptible to both PCN species. Maris Piper was the second most popular variety and accounts for around 13% of planted seed. Maris Piper is resistant to *G. rostochiensis* but susceptible to *G. pallida*. The variety Innovator was, with 1.6% the most planted seed potato with resistance to *G. pallida*. Arsenal was with the second highest level of cultivation representing 0.8% of the planted area, it also has the advantage of being dual resistant to both *G. pallida* and *G. rostochiensis*.

Initial PCN population/varietal tolerance

At higher initial populations of PCN, damage to the plants caused by the nematodes will limit the extent to which the PCN population will increase. The damage caused to the plant will also limit the commercial yield from the crop. Some cultivars which are highly tolerant of PCN damage, e.g. Cara, are capable of producing vigorous growth and maintaining yield at moderately high PCN populations. Such tolerant cultivars are also 'successful' in allowing PCN to develop to exceptionally high population levels. The PCN population is eventually self-limiting on all cultivars; if the density is high enough the host plant will die (Been & Schomaker 2006). Tolerant cultivars can withstand much higher PCN population levels than intolerant cultivars. Tolerance and resistance are not

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related values. Varieties can be tolerant and susceptible, tolerant and resistant, intolerant and susceptible and intolerant and resistant. Figure 4 below details the effects of tolerant and resistant varieties on yield and multiplication.

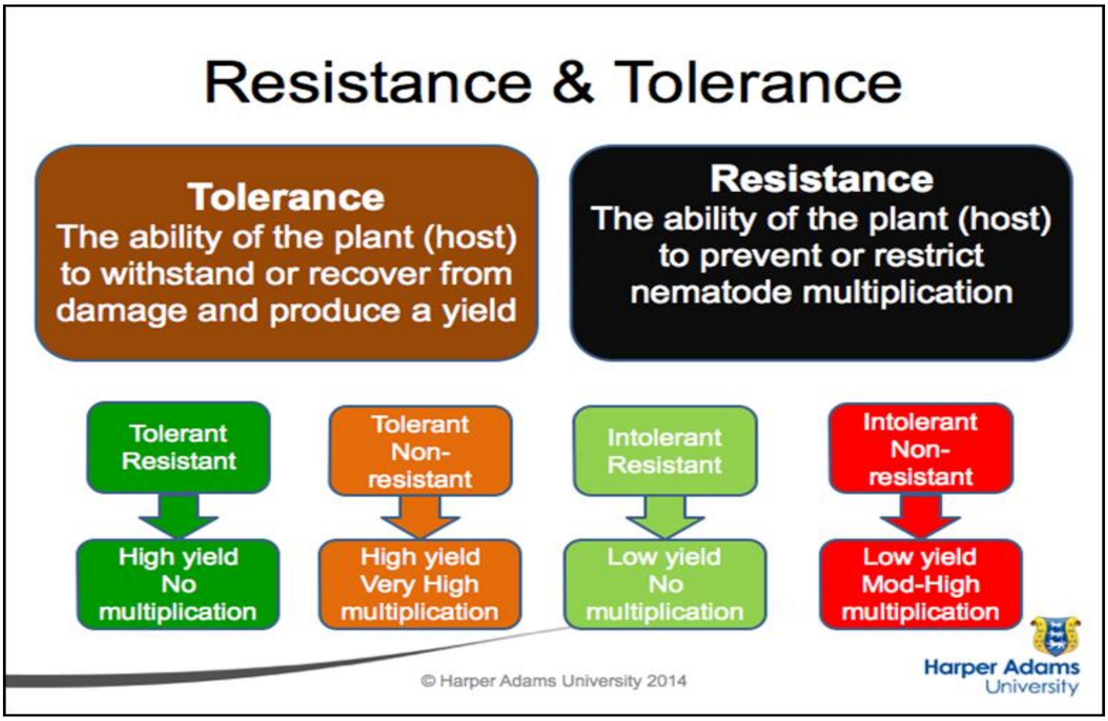

Figure 4: *Differences between tolerance and resistance courtesy of Ivan Grove (Harper Adams University)*

Figure 5: *AHDB SPot farm West where land has heavy infestation of* G. pallida*. Left panel Royal (highly tolerant), right panel Innovator (very intolerant).*

Decline rates, rotation length and groundkeepers

The rate of decline is well studied (Oostenbrink, 1950; Whitehead and Turner, 1998; Trudgill *et al*., 2014). Long rotations are often used as a tool for managing PCN, taking advantage of the natural hatch of c. 30% of the population that occurs each year in the absence of a host plant. Different studies have suggested vastly different decline rates:

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Devine *et al*. (1999) suggested that the number of viable eggs each year is reduced by about 10%, but in sandy soils decline rates of as high as 60% per annum have been recorded (Cole and Howard, 1962). This spontaneous hatch is generally cited as the reason for the natural decline of PCN in an infested field. This decline can vary with differences in soil composition, soil type and other environmental factors, including aeration and moisture, and is therefore difficult to predict. *G. pallida* is generally thought to decline more slowly than *G. rostochiensis*. Although populations will decline in terms of eggs per volume of soil, cysts can be very long-lived, so the decline is rarely matched in terms of cysts per volume of soil.

Taking advantage of decline rates enables rotation length to be used as a tool in the management of PCN. The idea behind this is the longer that land is left free from potatoes the further the population will have declined prior to the next crop being planted. This is however dependent on removing or killing all tubers from the field prior to the next crop being grown, any groundkeepers that remain will act as reservoirs for the nematodes which will continue to bulk up on these plants.

Nematicides and sterilants:

Nematicides are usually used at planting and have an effect on the juveniles emerging from cysts in response to chemical exudates from the roots of the growing potato crop. At the time of writing, the current granular nematicides are the oximecarbamate Vydate 10G® (10% oxamyl) from DuPont®, and the organophosphates Nemathorin® (10% fosthiazate) from Syngenta® and Mocap 15G (15% ethoprophos) from the Certis marketing company. Treatments can prevent early damage to root systems of the plants and help to achieve a healthy yield.

Nematicides reduce the initial population invading the plant roots, but those nematodes that are successful will still undergo a rapid multiplication on susceptible hosts. To manage the post-harvest PCN population within a field, a cultivar with resistance to the PCN present in the field should also be used. Sterilants and biofumigants act on the dormant eggs within the cysts to reduce the population. Minnis *et al*. (2004) showed significant ware yield increases from 22 to 35t/ha with granular nematicide. However, Trudgill *et al*. (2014) found that nematicides did not increase yield potential, but it was noted that this was very site dependent. Success rates for sterilants typically vary from a 50-90% reduction in the PCN population which is highly beneficial to the grower, particularly in reducing yield loss from a subsequent potato crop. At the time of writing, the liquid Metam 510 (Certis) which contains 510 g/l metam-sodium can be used in the management of PCN. The chemical penetrates the body wall of the nematode, interfering with essential enzymatic, nervous and respiratory systems leading to death normally within several hours. Its efficiency, like all nematicides is dependent on environmental conditions. Although it is only available in some parts of GB due to the specialist application required. As with granular nematicides, if the subsequent potato cultivar is susceptible to the PCN present, the consequent 50-fold population increase will more than negate any potential benefit in terms of PCN population management. There are very few nematicides available on the market and there is potential for stricter controls on these products to be placed in the future. This has stimulated interest in other potential control options, such as biofumigation and trap crops.

Biofumigants

'Biofumigation' is a term used to describe the suppression of soil borne pests, using natural biocidal compounds (principally isothiocynates). These are released in soils when glucosinolates in plant residues (predominantly brassicas) are hydrolysed. To

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provide the greatest benefit crops used for biofumigation generally have to be planted throughout the summer growing season for a period of approximately 12 weeks (Ngala *et al.*, 2014). Along with nematicidal effects some of the chemicals produced can inhibit hatching. The performance of biofumigation depends on which type of crop is used and a range of environmental and agronomic factors. These crops, while they may have some effect simply by being grown, generally need to macerated and incorporated immediately within the soil. The longer the optimal growing period of the crop the greater the potential benefit, therefore to get maximum benefit of these a summer crop would need to be sacrificed for their production.

Trap Crops

Trap crops come in two forms, firstly a potato crop, which must be harvested as soon as the first female is discovered on the roots of the plant. Thereby catching most of the nematodes within the plant prior to maturation. In some countries an early potato variety is grown to achieve control and a marketable crop. New data shows that both species of PCN are developing more quickly so local monitoring is required (Ebrahimi *et al.*, 2014)

The second and perhaps safer method of using a trap crop is to use a crop which is related to *Solanum tuberosum*. There have been several candidate crops studied for this and *Solanum sisymbrifolium* (sticky nightshade) shows the most potential to date (Dandurand, *et al*. 2014). In such circumstances root exudates cause the eggs to hatch and attempt to invade and feed on the host but they do not produce mature females or cysts. Trap crops have potential if they can stimulate a hatch of the eggs within the cysts without providing a host suitable for population increase. To date, trap crops are not widely adopted for use in Great Britain because they are generally adapted to warmer climates.

Biosecurity

Where land is PCN free, it is critically important to keep it that way. Cysts are sedentary and cannot move by themselves. The most likely way for them to spread is through the movement of soil. Therefore the most effective way to prevent their spread is through preventing movement of soil. This includes from machinery, from the soles of shoes, from planting material, from animals.

3. Materials and Methods

3.1. Soil Sampling for PCN

The details of the work are provided in a separate report "PCN Soil Sampling" and are not repeated here.

3.2. PCN training courses 2016

A course organised by Kim Davie, Yvonne Cole and Mairi Carnegie took place on the 23/24th August and 13/14th of September 2016. *Heterodera* and *Punctodera* cysts were sourced from ADAS to add to cysts collected at SASA. PCN cysts were sourced from SASA's reference collection, Ro1, Pa1 and Pa2/3 were all used. Non-viable cysts were either sourced from known fields with long dead populations or left over cysts from trial work. Other confusers such as seeds and microcysts were also recovered from visual samples for use during the course.

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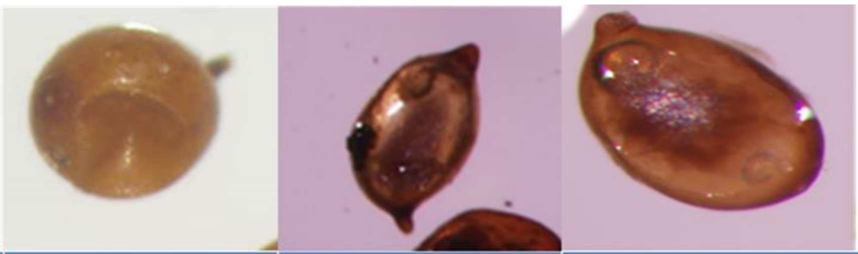
The course consisted of an introductory presentation to cyst forming nematodes detailing the key features of all cyst forming nematodes known to be present in GB. Participants were provided with a workbook detailing all of these key features. There was a guided tour of the nematology facilities at SASA and an explanation of the method we used. The rest of the course was laboratory based and participants were initially shown how to set up and use the microscopes. Each of the cyst genera were taught in detail while participants examined and dissected specimens looking at key features, both on stereo and compound microscopes. Key features were pointed out as shown in table 4.

Table 4: *Key features of cyst forming nematodes*



Feature	Globodera	Heterodera	Punctodera
Cyst shape	Round shaped	Lemon shaped	Ovoid shaped
Pattern	Wavy pattern	Zig-Zag pattern	Finger print pattern
Cone	No cone	Cone present	No cone
Vulva	circumfenestrate	Ambi or bifenestrate	circumfenestrate
Anus	V shaped	Difficult to see sometimes V shaped	circumfenestrate
Bullae	Absent	Often present	Absent

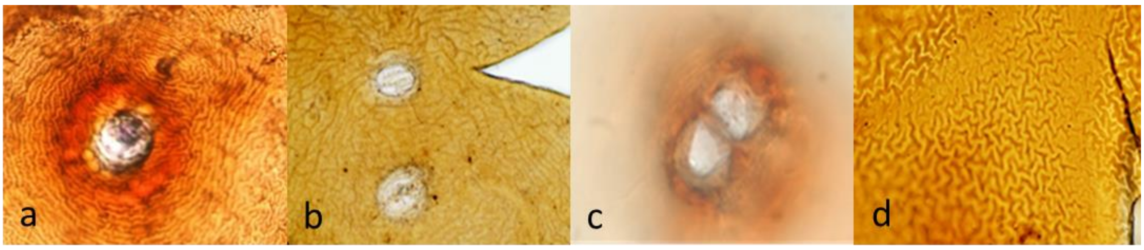


Figure 6: *Vulval region of (a)* Globodera*, (b)* Punctodera *and (c)* Heterodera *all shown,* Heterodera *displaying bullae and (d) picture of* Heterodera *cyst wall patterning.*

After the features of each of the different cyst genera were shown to the participants they were given slides with mixtures of cysts in order for them to segregate out *Heterodera*, *Punctodera*, *Globodera* and non-nematode material such as microcysts or seed cases. Once these had all been successfully segregated by the participants, filter papers which had been seeded with different combinations of cysts were provided for detection and examination. Several hours were spent on these two exercises as these were the most important for learning the new information about features that they had been taught.

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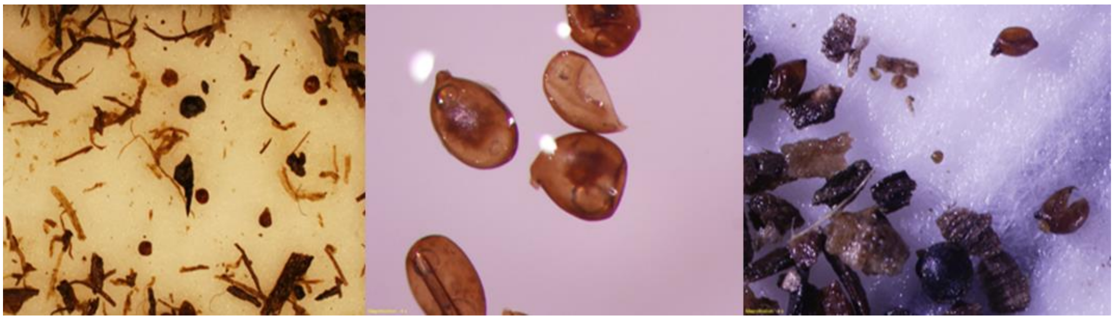

Figure 7: *Cysts on slides and on filter paper. Left pane PCN, center pane* Punctodera *and right panel* Heterodera*.*

In addition to this training participants were shown the important features for speciation of *Globodera* cysts, using juvenile worms to determine whether *G. pallida* or *G. rostochiensis*. These features include shape of basal knobs along with stylet length and knob width as shown in figure 8. .

Figure 8: Globodera pallida *juvenile worm*

3.3. Proficiency testing

Following the training course the laboratories were sent a series of samples to determine how well they had taken on the training that they received. The first aim was to test the laboratories' ability to distinguish between different cyst nematodes following the training course. The second aim of these samples was to give laboratories the opportunity to distinguish the potato cyst nematode cysts as being either *G. rostochiensis* and *G. pallida*. Only a few laboratories provide a speciation as service but morphological identification to species was included in the training course at SASA therefore all laboratories were given the option to participate in this test.

Initial follow up test – January 2017

Four Eppendorf tubes containing cyst nematodes were provided to all laboratories.
The samples were seeded as follows:

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Table 5: *Composition of cysts per Eppendorf – initial follow up test*

Sample Number	Total number of cysts	Number of PCN	G.rostoch	G.pallida	Heterodera	Punctodera	Non viable
A	10	10	9				1
B	10	8	3	3	2		2
C	10	6	2	4	3	1	
D	10	6		6	2	2	

After this initial reassessment laboratories agreed to continue with a self-sustaining proficiency test, which would initially be prepared and distributed by SASA staff.

Proficiency test - September 2017

Aim 1 – to test the ability to distinguish PCN from other cyst nematodes

- 5 eppendorf tubes per laboratory each containing different compositions of cysts; laboratories were asked to determine which genus each cyst belonged to.

Aim 2 – Speciation – To test ability to distinguish between *G. rostochiensis* and *G. pallida*

- The PCN cysts from aim 1 were used for testing the ability to distinguish between the two species of PCN.

For the above aims, five Eppendorfs containing cyst nematodes were provided to all laboratories. The compositions of cysts in these Eppendorfs are detailed in table 6 below. The *G. rostochiensis* used were grown in 2014 (SASA Ro1 population) and the *G. pallida* were grown in 2013 (SASA Chavornay Pa2/3 population). Cysts were not returned to SASA for confirmation because in order to carry out speciation cysts would have to be destroyed.

The samples were seeded as follows:
Table 6: *Contents of Eppendorf tubes for identification*

Sample Number	Total number of cysts	Number of PCN	*G. rostochiensis*	*G. pallida*	*Heterodera*	*Punctodera*
A	10	0			6	4
B	10	5	3	2	3	2
C	10	8	4	4	2	
D	10	5	5		4	1
E	10	10		10		

Aim 3- Cyst recovery from soil samples

The aim of this set of samples was to establish how effective each laboratory is at extracting PCN from soil samples. This was achieved by providing soil samples (1 soil type) seeded with a known number of PCN cysts. For the soil samples, soil was collected from a field on SASA's farm which has been intensively sampled and is known to contain no PCN. Samples sent to the laboratories were 400ml samples, an average dry weight of these samples taken at SASA was 444g. A wet weight and a dry weight of 400ml of this soil were worked out to be used for comparison (See table 7 below).

Aim 4 to distinguish PCN from other cyst nematode species in float material

- Along with PCN cysts in aim 3, a known number of *Punctodera* and *Heterodera* cysts were added to the soil samples and these results recorded.

Aim 5 – quantification of PCN in soil samples

- Participants carried out egg counts on cysts extracted from the soil samples circulated for aims 3/4
- There will be inherent variation in egg numbers between cysts. Therefore, SASA counted the number of eggs/cyst in a sample of the cysts circulated to participating labs. This gave an indication of the variation in egg numbers and this was taken into account in the analyses.

Table 7: *Average sample weights*

Sample	Wet weight (no tray)(g)	Tray weight (g)	Dry Weight (inc. tray)(g)	Actual Dry weight (g)	Water weight lost (g)	% Weight lost
1	539	142	617.2	475.2	63.8	11.84
2	485.6	140.2	563.8	423.6	62	12.77
3	478.9	141.8	560.2	418.4	60.5	12.63
4	509.5	140.2	585	444.8	64.7	12.70
5	524	141.8	599.1	457.3	66.7	12.73
Averg. =	507.4	141.2	585.06	443.86	63.54	12.53

The cysts used for the soil sampling test were Pa1 grown in 2015 from 2014 stock population. Different compositions of cysts were created as detailed below. A hole was created in the soil sample, the cysts were deposited at the bottom of the hole and covered in soil to ensure all cysts remained within the sample.

Table 8: *Soil sample cyst quantities*

Sample number	Number of PCN cysts per 400ml	Number of Heterodera cysts per 400ml	Number of Punctodera cysts per 400ml
A	2	8	0
B	4	0	0
C	12	4	4
D	0	8	2
E	14	2	0
F	40	0	0
Total number required per lab	72	22	6
Total number required	504	154	42

The soil samples and the Eppendorfs (within a larger sealable tube) were placed in cardboard boxes for sending to laboratories. A meeting was held with representatives of the laboratories on 21st March 2017. It was agreed that a randomisation step (i.e. random allocation of samples to participants) would also be included so that someone not involved in the generation of the samples would allocate the samples randomly to different participating labs. To randomise which laboratory got which samples, compliment slips were written for each laboratory and then turned upside down for a member of staff to pick (who didn't know what for) each slip was then placed by order selected in each box from one to seven. Parcels were then sent by recorded delivery to each laboratory on 5 September 2017, participants were given until 20 October to complete the test and return the results.

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The results of the test were provided in an anonymised form to the participating labs. At the outset it had been agreed that the details within the report would not be disclosed outside the proficiency testing group.

3.4. Farmer interviews

For a period of six weeks over the summer 2017 the post holder worked closely with a Dutch student who was given a project to compare the differences between the PCN situation in Scotland and in the Netherlands. The aim was to compile as much information about the varieties used in both countries and to determine any cultural differences in attitudes to PCN and PCN management.

During the period of the internship interviews were arranged with 18 farmers covering the following geographical range which includes the main seed potato growing areas which were easily accessible from SASA.

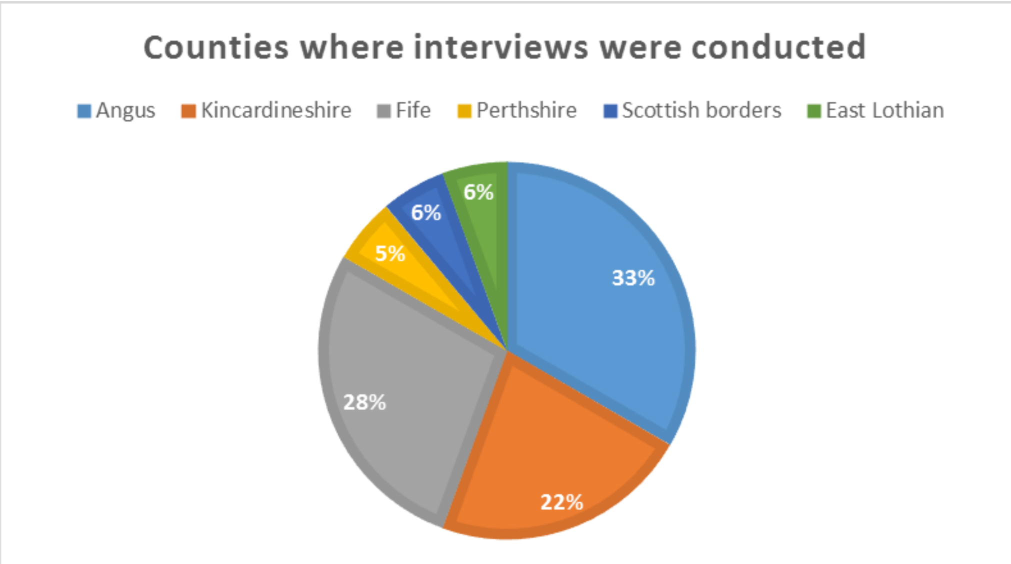

Figure 9: *Breakdown of areas where interviews were conducted*

We obtained a list of farmer details from the seed potato classification scheme incorporating a range of ware and seed producers. Growers, producers and breeders were all contacted and interviews arranged with them. Interviews were arranged by telephone and each visit lasted approximately an hour depending on the grower.

Growers were asked the following questions:

- Where is the farm?
- On how many hectares do you cultivate crops?
- What kind of crops do you have in your rotation plan?
- Why the choice for these crops?
- Do you have a solid/permanent rotation plan?
- What do you think is the most damaging pest or disease affecting potato production?
- What kind of potato varieties do you grow?

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- Why the choice for these varieties?
- What is the most important factor in choosing a potato variety?
- Are these varieties resistant against PCN?
- What would be the main reason for not choosing resistant varieties?
- Is *Globodera rostochiensis* a problem on your farm?
- Is *Globodera pallida* a problem on your farm?
- What is your advice for growers with PCN problems ?
- Would you try to cultivate resistant potato varieties to *Globodera pallida* and why?
- Are you pre-cropping sampling to determine species?
- How often do you take soil samples?
- Do you cultivate cover crops? And if yes, what kind of and for what purpose?
- Where does the soil go that is left over from the sorting machine?

Interviews were conducted with two interviewers, one who asked the questions and the other who noted down responses. Results were recorded and charts produced from the answers. Separate interviews were conducted with producers and breeders but this was more of a general discussion about the breeding process and what factors were important to producers in a variety. We also interviewed two breeding companies and five of the major potato producers in Scotland.

3.5. Additional Objectives

Molecular identification of PCN

At the beginning of the project the fellow was shown how to carry out molecular identification of PCN and all of the steps in the process involved. She carried out molecular identification under the supervision of experienced diagnosticians. DNA and PCR reagents were prepared and plated into a 384 well plate using a Hamilton starlet robot prior to performing a real time PCR using an Applied Biosystems 7900 SDS HT machine. Results were analysed using SDS software which gave the outputs of the reaction. A cut off of a CT value of 34 was used to determine whether samples were positive or negative.

Identification of cyst nematodes

The fellow had several years of prior experience in the morphological detection and identification of cyst nematodes, therefore no formal training was provided in this subject at the time. However, as a refresher the first task of the post holder was to complete the proficiency test provided by ADAS through AHDB potatoes. There was also opportunity for the fellow to be involved in visual analysis of statutory samples, export consignments and trial work throughout the project in order to refresh and maintain the specialist nematology taxonomy skills in cyst nematode identification.

Identification of free living nematodes

The fellow had previously completed training in the identification of free living nematodes to genus level. Additional formal training in the identification of relevant free living nematodes to genus was completed through a week long course provided by

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Professor Gerrit Karssen, an internationally renowned nematologist. This covered the major plant-parastic nematodes species within the Tylenchomorpha (root-knot, cyst, lesion, leaf and bulb/stem nematodes) along with the virus transmitting nematodes of the Triplonchida/Dorylaimida. Species were studied through the aid of lectures, keys and reference specimens. The fellow obtained some soil samples from SRUC which had been submitted for free living nematode identification and this gave the opportunity to study some of the species relevant to potato production. The post holder also provided assistance in the identification of *Ditylenchus spp*. from bean and bulb samples and a range of plant parasitic nematodes (potentially quarantine) from bonsai imports.

Understanding the Scottish statutory system

The fellow had involvement in all parts of the statutory testing process and is currently undertaking an improvement project to gain efficiencies throughout the testing process.

Review of current PCN management practices

One of the first roles within this post was to be involved in an AHDB Grower Guide for PCN management. This provided the fellow with valuable information in to the different management options available to growers. The project to interview farmers, breeders and producers provided valuable insights in to current PCN management practices and how they are being applied in the field.

Review of biosecurity measures

The fellow assessed biosecurity risks in spreading PCN from one farm to another and produced guidance for this in anticipation that this can be produced in to a leaflet for growers.

4. Results

4.1. Farmer Interviews about Management Practices

The results detailed below are from the 18 farmer interviews that were carried out, rather than from discussions with breeders and producers although this information will be included within the discussion section. The farm sizes ranged from less than 100 ha to several thousand hectares. In 3 cases the farm produced ware only, in 7 they grew both ware and seed and 8 were dedicated seed growers.

Rotation plans

Most of the rotation plans alternated between cereals and potatoes, with growers tending to go for a 1 in 6 to 1 in 8 rotation. Two growers used longer rotations of 1 in 10 for seed potato crops. In general they told us that rotations were mostly for the potatoes as this was the main cash crops. Other crops were chosen on the basis of the returns made from these, cereal crops tended to go for alcohol production rather than human or animal feed. In addition to cereals other crops planted included oil seed rape, carrots, turnip, broccoli and flower bulbs. A couple of growers also rented land out for bean and pea production as these were specialist areas that had good rental returns. Several growers also kept cattle and for this reason had grass in the rotation for a few years at a time. One grower also had Christmas trees.

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Variety choice

When asked about their choice of variety most growers said they had little to no choice in which varieties they planted. They only grew what they were asked to produce through a contract with a producer. Most liked the guaranteed income they got from going with contract but understood that this meant they had little choice in variety or quantities of varieties. Of those that did not go on contract they tended to grow Maris Piper or other free, traditional multi-purpose varieties as they could always find a home for them if they are versatile. Growers tended to stick with varieties they knew well where possible.

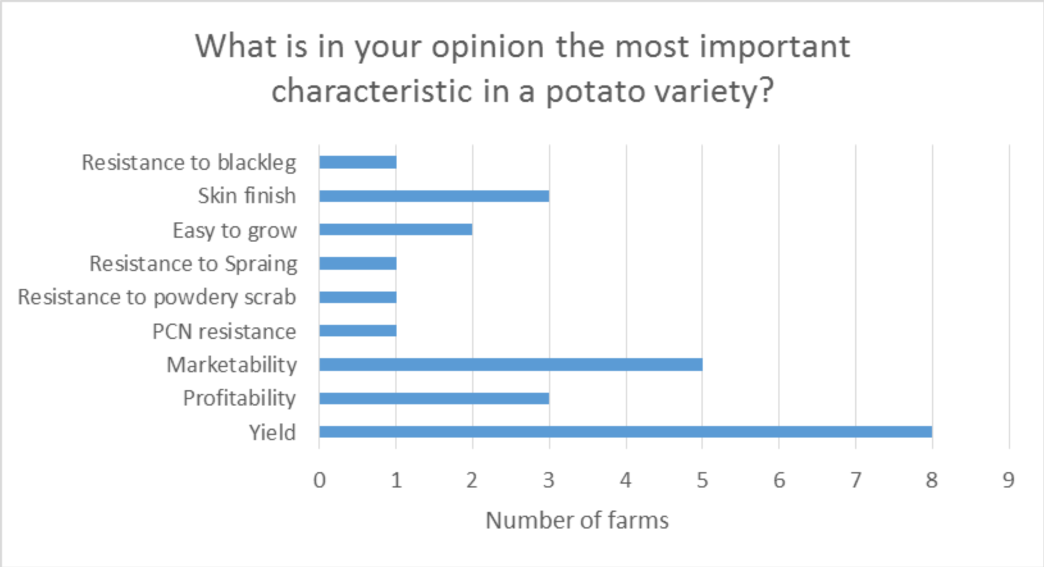

Figure 10: *Chart showing farmers most important variety characteristics*

When asked what they saw as the most important factor in a potato variety, yield and marketability were rated as the two most important factors. A few growers said that skin finish was of critical importance especially for packing varieties. Overall most were worried about the return from the variety so it had to do well in local conditions and had to make money.

Most damaging pest or pathogen

Growers were asked what they believe to be the most damaging pest or pathogen. The results are shown in the graph below, some growers gave two pests/pathogens as being equally important.

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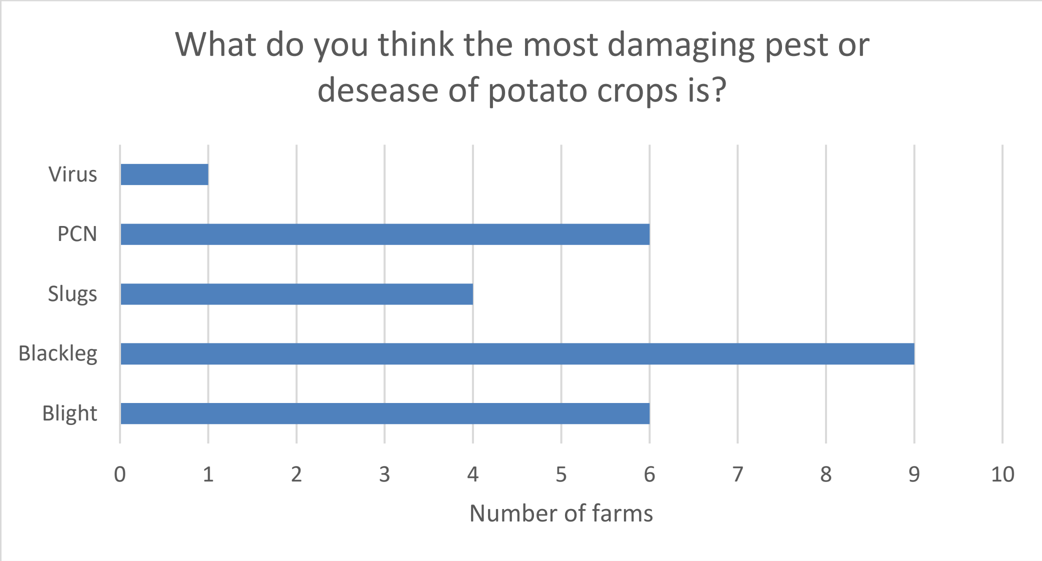
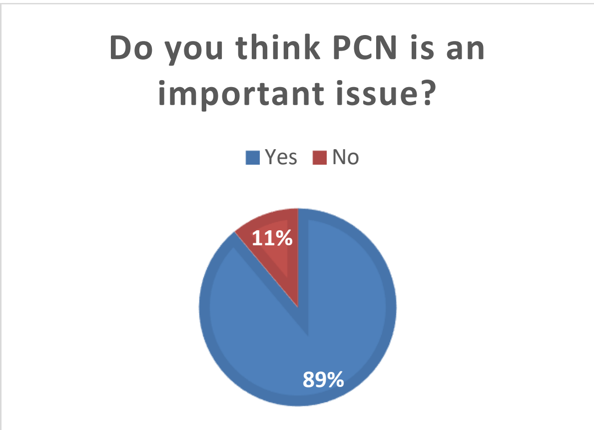


Figure 11:*Chart displaying farmers opinions of the most damaging pest or pathogen*

Currently most of the downgradings of potato crops in Scotland are as a result of blackleg and therefore this is at the forefront of grower's minds and ranked as the most important issue accordingly. Blight and PCN were jointly the second biggest concern. Growers told us that they were very concerned by blight especially those strains that were becoming resistant to the fungicide treatments available. Blight is well managed at the moment but it is costly to treat for, and if for any reason it would not be possible to treat for this it would become the biggest concern. The farmers that cited PCN as the greatest concern all had PCN and were taking steps to manage it, or had lost land to seed production as a result of it. The growers we spoke to who had not had positive PCN tests were not as concerned about it. Virus is also costly to control but not so much of an issue in Scotland, only one grower thought this was the most damaging although this was related more to its potential threat than current situation.

Most of the growers we interviewed believed that PCN was an important issue and were worried about land being lost to PCN, although a few were not sure if the problem was getting worse or if it was just that SASA was testing more soil. The biggest concern about PCN was loss of land availability for seed rather than the affect that PCN may have on yields.



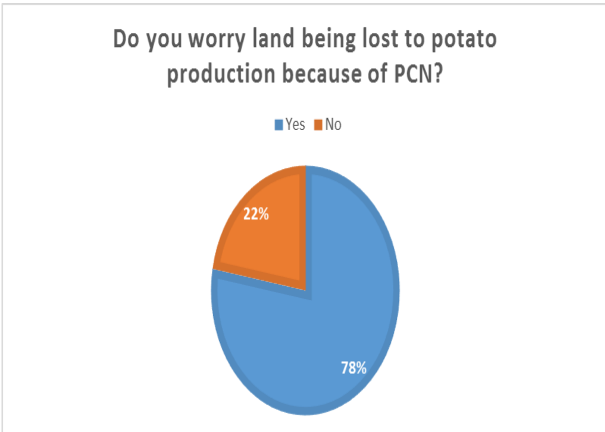


Figure 12: *Pie charts detailing level of concern by farmers about PCN*

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Ware growers in general were less worried about PCN as they do not need to test their land. One ware grower we spoke to told us that they used to grow seed but a few fields came back as infested which prevented the use of the land and therefore it was considered safer to grow ware, as it was believed there was no need to test for PCN. Returns seem to be roughly the same for seed and ware and therefore there are no strong incentives to remain in seed production. A seed grower we spoke to said this was a problem for him as if he tested the land and it was found to be positive the land owner would rent the field to a ware grower instead and thereby they were losing a lot of potential land. Land owners who do not grow potatoes are not very aware about the long term risks of PCN and will sometimes prefer to rent land to ware growers as they do not need to test for PCN, and therefore are unlikely to pull out of any rental agreement.

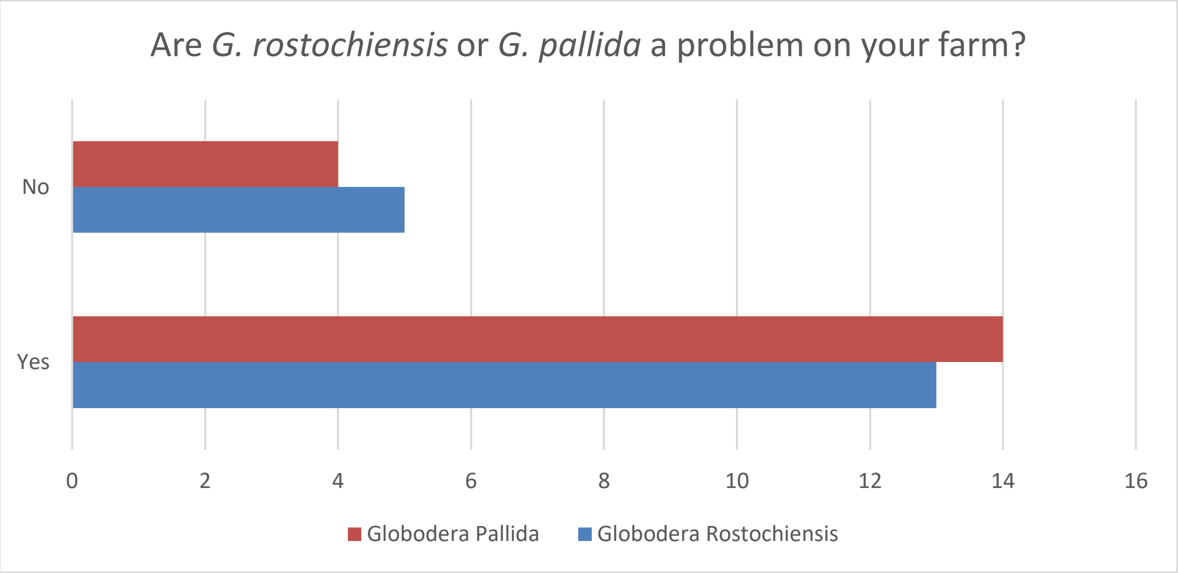

Figure 13: *PCN presence on farms visited*

Most of the farmers we spoke to did have PCN on their land, most of the farms had both species, a couple of growers said they did not know which species were present. In total only 3 farms claimed not to have any PCN findings.

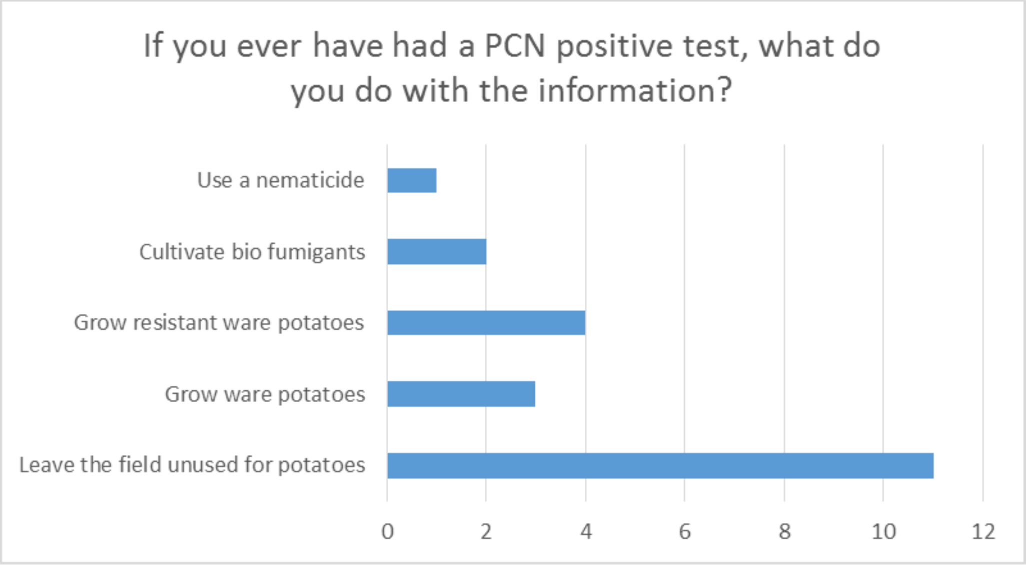

Figure 14: *Chart detailing action taken by growers where there was a positive PCN result*

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Most of the seed growers that we spoke to said that if there is a finding of PCN they will take the land out of seed production and test again after another rotation. A few said if *G. rostochiensis* is present then they will plant a resistant variety such as *M. Piper* to bring down the population. In some cases the farmer would grow ware under a control programme, this tended to be more on rented ground where they had already secured a rental agreement for the land which they did not want to lose.

In general growers were aware of resistant varieties and how these differed for the two species but not all.

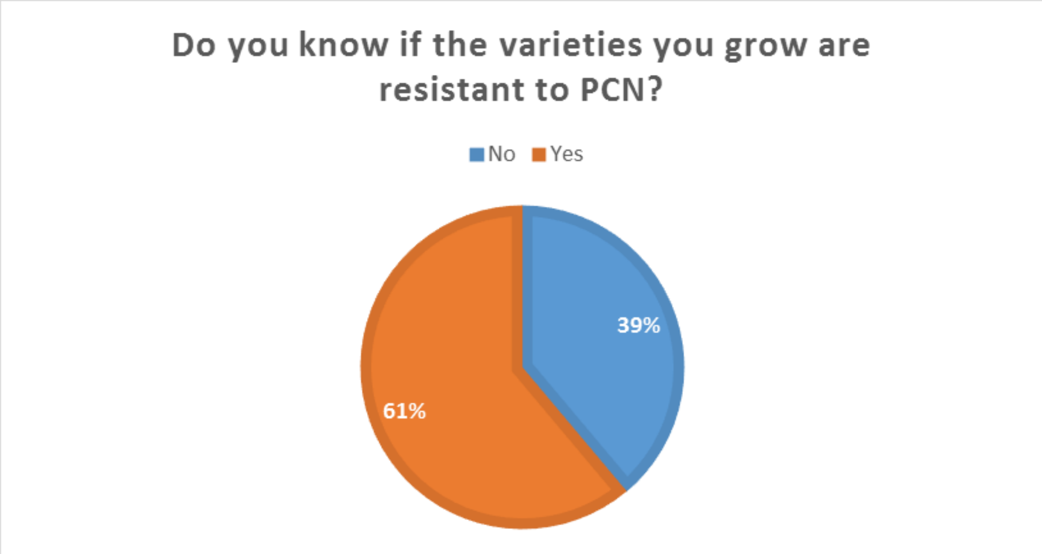

Figure 15: *Pie chart showing grower knowledge about the varieties they were planting in terms of PCN resistance.*

Not all knew if the varieties they were using were resistant to PCN, this was particularly true for those which were grown on contract. Where growers were producing varieties that were resistant to *G. pallida* they were not necessarily using them to maximum effect. We were told that they tend to grow the same cultivars in the same fields on each rotation. This has two advantages for the grower, they know which cultivars do well on which soils and exactly how best to optimise the yields in these fields. In addition to this any groundkeepers will be the same variety when the next crop is grown which will prevent mixing of varieties. Although it is possible to see how this is advantageous to the grower it does not promote best practice in controlling groundkeepers. Groundkeepers are an important reservoir for potato cyst nematodes and therefore should always be controlled. Most of the growers would like to be able to use cultivars with resistance to *G. pallida*, the ones that said they didn't think it necessary were the same as the people who didn't think they had a problem with PCN at the moment. All other growers would like to be able to use varieties which are resistant to *G. pallida*.

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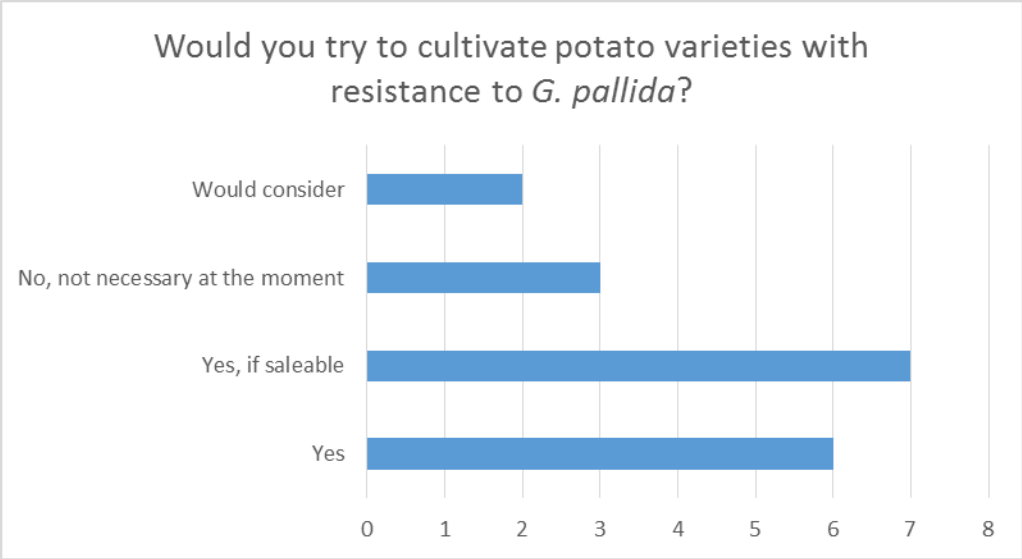

Figure 16: *Chart showing farmer responses to whether they would grow G. pallida resistant varieties*

Only a couple of the growers we spoke to actively sought out and used *G. pallida* resistant cultivars in rotation on their farm to serve the purpose of controlling populations and alternated these with susceptible varieties. The rest would like the option of using resistant varieties but think there are serious limitations with the ones available. One of the producers we spoke to told us that for ware the furthest North that it is possible grow potatoes for processing is Yorkshire. Almost all of the potato varieties that are available with high levels of resistance to *G. pallida* are processing varieties, for this reason there are almost no cultivars that can be used for ware in Scotland.

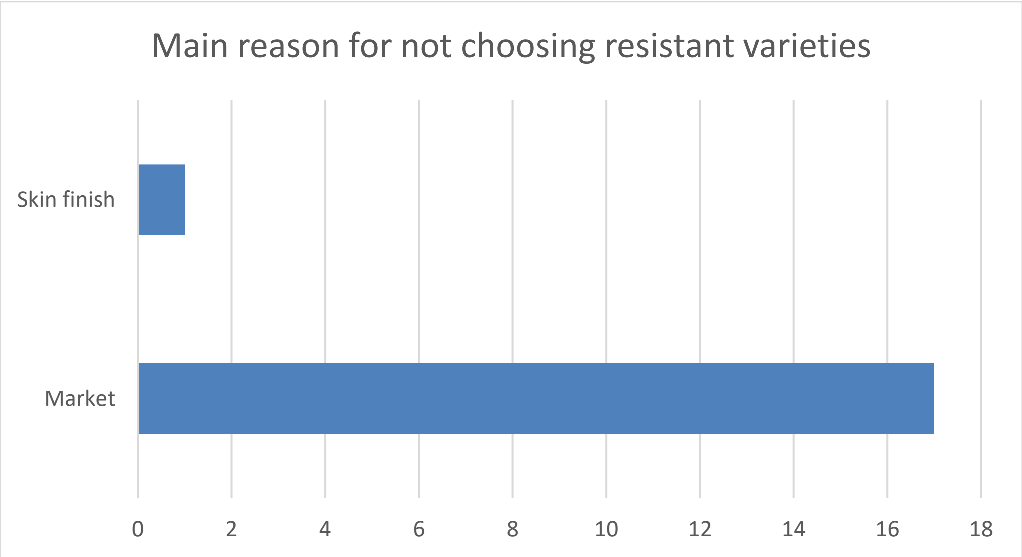

Figure 17: *Chart showing main reasons given for not choosing resistant cultivars*

The vast majority cited marketability as the main reason for not using resistant cultivars. As stated previously most growers are tied in with producers and grow on contract which allows them a guaranteed income. Producers generally have their own varieties which have different qualities. Only one of the producers we spoke to have a resistant variety with a score of 8/9 for *G. pallida* available to their growers, however this variety was largely being replaced due to its poor tolerance to PCN.

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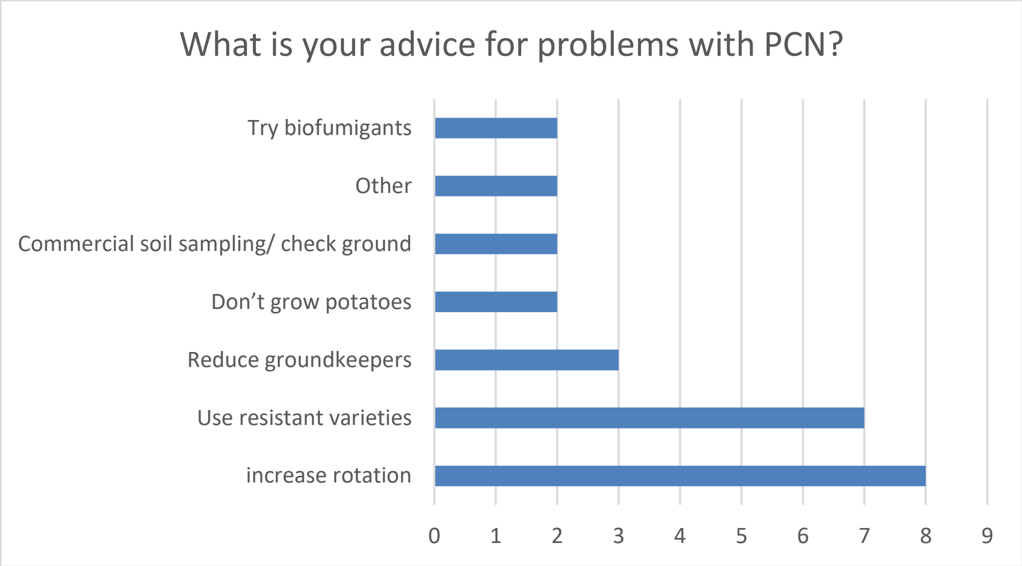

Figure 18: *Chart showing the advice farmers would give for PCN management*

We asked farmers what they believed was the best way to tackle problems with potato cyst nematodes. Most believed that increasing the length of rotation or using resistant varieties were the most effective tools available to manage PCN. Other suggestions were to not grow potatoes in infested land, to effectively control groundkeepers or to try biofumigants.

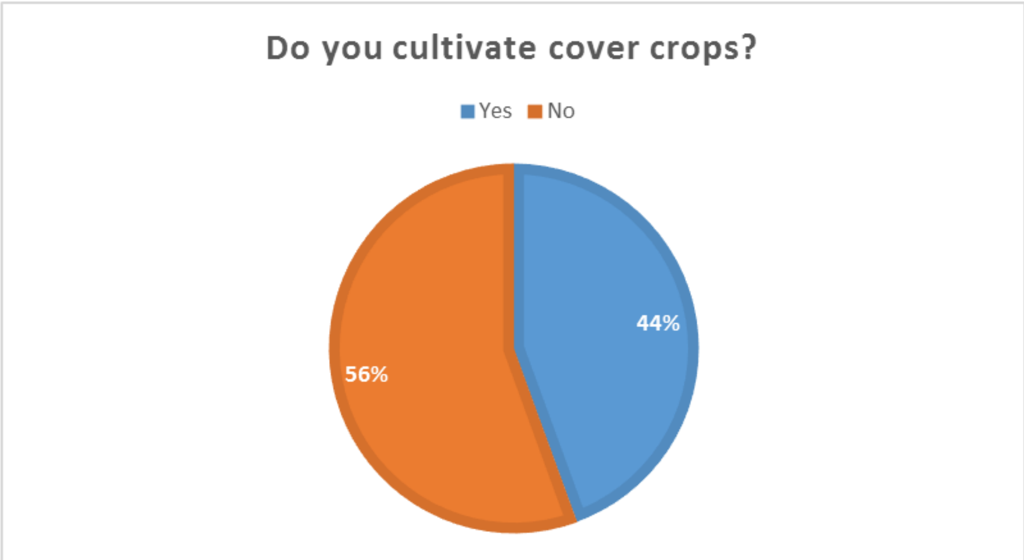

Figure 19: *Chart showing use of cover crops*

The main reason for cultivation of cover crops cited by growers was for soil structure, a couple were using them in an attempt to reduce the PCN population.

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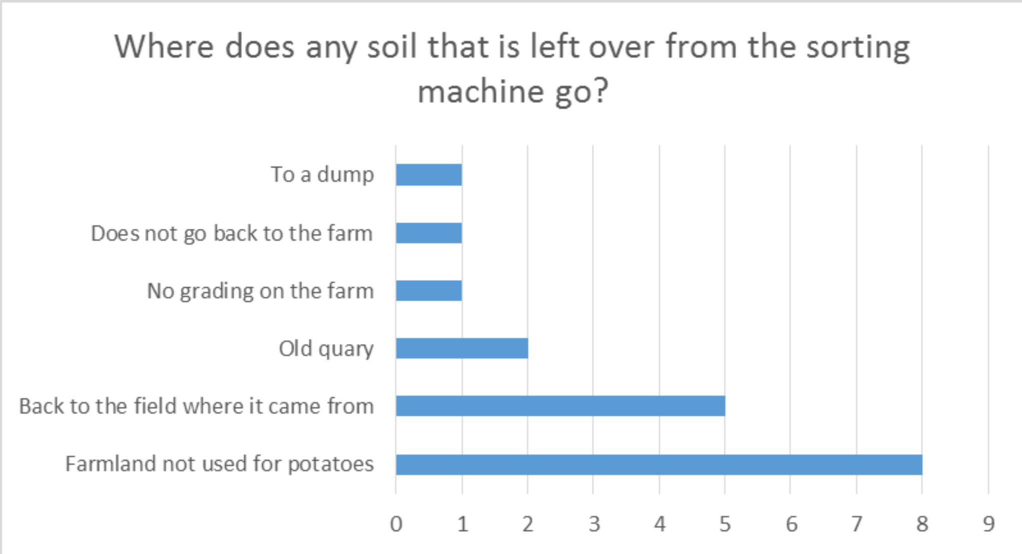

Figure 20: *Chart detailing what happens to waste soil from graders*

Some of the growers raised concerns about soil that was returned to them if grading took place off of the farm, soil returned is supposedly from the same field however we were told of an incident where the soil returned was a different colour to the soil on their farm. Since then they have refused to accept soil back. Most of the growers did not return the soil to the field where it came from but had another site where it went to that was not used for potato production.

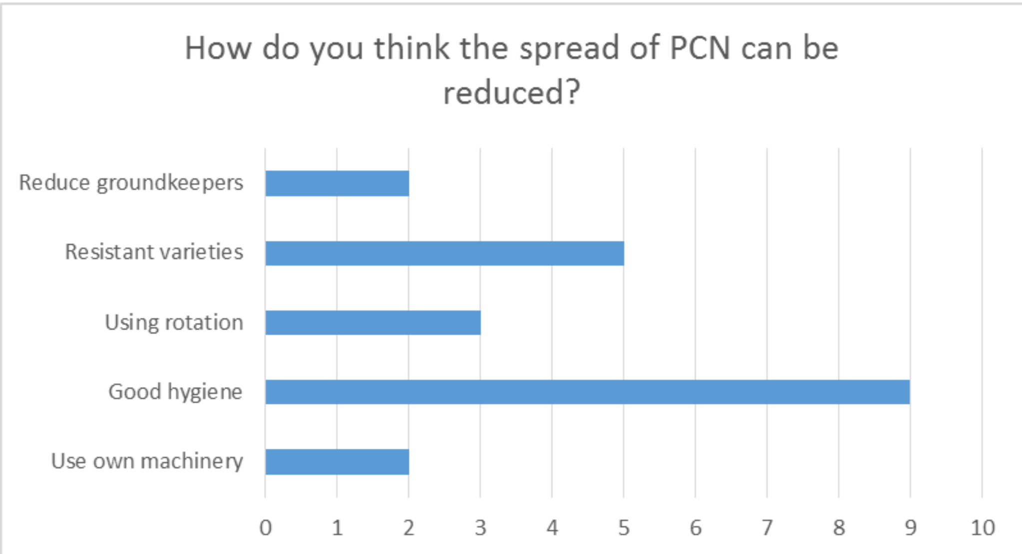

Figure 21: *Chart showing responses to question on how the risk of spreading PCN could be reduced.*

To reduce the spread of PCN, most growers thought that the most important factor was good hygiene and biosecurity, this included not sharing machinery. Most of the growers we spoke to use their own machinery, but the ones who used contractors were concerned about the levels of cleanliness. They did tell us that although it would be preferable to clean machinery between fields this is not always practical.

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5. DISCUSSION

5.1. PCN Training Courses and Proficiency Testing

There were initially concerns raised about the standard of laboratory diagnostics in GB and one of the key roles of the post holder was to bring about improvement in this area. The training courses held at SASA in 2016 received excellent feedback, where all laboratories involved reported them to have been useful. Laboratories were taught how to detect and identify a range of cyst forming nematodes using morphology. Overall the results from the proficiency testing demonstrate that some laboratories have performed consistently well in both the SASA and ADAS proficiency tests, and other laboratories have made improvements in their diagnostics of cyst nematodes. In the ADAS proficiency test the contents of 16 out of 35 tubes were identified correctly by all 7 laboratories and in the latest proficiency test set by SASA the contents of 33 out of 35 tubes were identified correctly.

Overall the results of the September 2017 proficiency test were very good with almost all of the laboratories correctly identifying the genera of cysts present. One laboratory did identify other cysts as PCN in a couple of the samples. Out of the five laboratories that attempted speciation, two laboratories made errors in the identification to species on a couple of occasions, however all other results were good. All laboratories demonstrated the ability to effectively extract PCN cysts from soil samples. Laboratories identified different cyst genera present, but in some of the samples laboratories only found PCN where there were other cysts present. This is probably mainly a consequence of sub-sampling. Variation within the egg counts is to be expected as the quantity of eggs that are present in each cyst varies. Most of the results were within an acceptable range from the expected population.

Overall, detection of PCN from soil samples was good, however a few laboratories which used sub-sampling did not always detect other cyst nematode species that were present in the sample. Where other cyst species were found that had not been seeded, it is likely that PCN had not been correctly identified and laboratories should consider how to improve their ability to correctly identify all PCN . The quantification objective was well met, however sub-sampling will have an impact on the accuracy of these results and laboratories should consider if the level of sub-sampling and the method of mixing samples is always suitable. For one of the laboratories, the cyst count was close to expected values but the egg counts per gram did not match up. This laboratory should consider the method it is using to achieve egg counts and how this can be improved. To maintain the clear improvements that have been made, I recommend a regular programme of self-assessment audits to be carried out between the laboratories. There is also scope to include further training courses if deemed necessary. Laboratories may also wish to consider whether they want to publicise their commitment to ongoing proficiency testing and the programme of continual improvements that are now in place.

5.2. Farmer Interviews

Critical review of current PCN management practices

From discussions with growers we were able to establish some of the motivations in the management of PCN at a practical level. Most of the growers were concerned about the loss of land to PCN particularly in the future but the general feeling was that this was due to the restrictions the government place on this land and not because they were

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worried about the effects of the PCN infestation. Where fields or part of fields are given a clear result, often the current belief is that there are no PCN in this part and therefore there is no reason to manage PCN in this land. Detection of PCN is dependent on the quantity of cysts present, the size of the soil sample and the sampling protocol. Therefore, a clear soil sampling test at 400ml does not necessarily mean that there are no cysts present within that section of a field. This appears to be an important knowledge gap for growers and where increased education is required.

The most effective tools available to manage PCN infestations are resistant cultivars of potato. These have been highly effective at supressing *G. rostochiensis* and are an integral part of any control programme. Varieties resistant to *G. pallida* only account for 3% of land grown in Scotland. Many growers are tied in to contracts with potato producers as this allows for a guaranteed income. Potato producers usually know who they can sell their products to, or they produce their own to meet market demands. Of the different producers that we spoke to only one was growing a *G. pallida* resistant variety.

All growers and all producers said the most important factor in a potato variety is marketability, PCN rarely comes in to their decision making process. This leaves the management of PCN using resistant varieties very challenging. At the moment, almost all *G. pallida* resistant varieties are processing varieties. In Scotland the climate does not allow tubers to produce the correct starch and sugars required for processing so these varieties can only be produced as seed and only in the quantities that the producers are able to market. The other factor to take in to account about using land for potato production is that some varieties do well on some land and other varieties on other land, growers work with agronomists to decide the best varieties for their own conditions. Many of the farmers we spoke to did not know whether or not the varieties they were growing were resistant to PCN with a few notable exceptions. They were usually choosing varieties based solely on the marketability and most of them were on contracts with producers, some with more than one producer meaning they had little say in the varieties they grew.

The demand for certain varieties seems to be driven by the end processor or seller e.g. the supermarkets, crisp companies, frozen food manufacturers or fast food restaurants. They have certain requirements for their products, supermarkets key concerns are skin finish and general appearance along with being general purpose cooking varieties. Processing varieties are concerned with shape for crisps or chips and with starches and sugar content, fry-ability etc. For ware Maris Piper is the most popular variety because of its versatility which allows it to go for either processing or the fresh market. In seed Hermes is most popular because of its popularity in Egypt. In order to have any influence over which varieties are being grown in the field we need to target the end market and producers rather than the growers.

Current attitudes in Scotland could be counterproductive in the management of PCN. Some farmers, particularly where there is a problem with PCN have taken the approach that because the land they have may fail for PCN in the departments' test that they will not take the risk of the land being recorded which would prevent them growing potatoes. They have taken the decision to instead focus on ware production only and will never test the land for PCN, they make no consideration of the variety they use other than marketability. This will allow PCN populations to grow unchecked, and essentially gives up the land for seed production indefinitely. The value of ware is similar to the value of seed but doesn't have the same restrictions. When renting land, landowners may

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preferentially rent to ware growers because there is no risk that the renter will pull out due to positive PCN test. Ware growers are often willing to pay more rent than seed growers. Ware varieties in Scotland have to be table varieties and the market for this is decreasing, so a shift to ware could be detrimental to the longevity of the potato industry in Scotland.

The best soils for growing seed potatoes in Scotland are located in Angus however a lot of this land is being lost to ware due to the increasing PCN situation, many of the pack houses for supermarkets are also in Angus to be close to the source of ware potatoes and the value of ware potato and ware potato land is so high. Many of the seed growers for this reason are moving further afield looking for land to rent this means that there is a risk of machinery and soil moving around the country to previously clean areas.

While rotations can be an effective tool in managing PCN a few of the wheat fields we observed when we were driving around the country had large potato component from groundkeepers (Figure 22) . There appears to be a particular issue where land is rented that the landowner is not concerned about potatoes in the crop and ploughs them in to the ground when the land is returned to them. Added to this a lot of farmers used to leave potatoes on the surface of the ground in order for the frost to kill them in the winter, the winters now however are a lot milder so the tubers are not being killed off in the same manner. Unless groundkeepers are controlled, long rotations are broadly meaningless.

Figure 22 : *Winter wheat field with large groundkeeper problem*

Another potential risk area identified is that cows are often fed ware potatoes or carrots and other root vegetables where there is soil attached. The slurry from the cows is used to fertilise the land including seed potato land, more work would be required to establish the risk to the seed crop from PCN through this route. From personal discussions at the European Society of Nematology meeting in 2018 it is believed that cyst nematodes can survive through the guts of cattle but are killed due to the heat of the midden. This could be an area for further investigation.

Where potatoes are graded offsite using producers' facilities the excess soil is often returned to the farm, this can potentially be soil from a different farm and there is no way of knowing exactly where this came from, most growers have somewhere to put this soil that will never be used for potato production, but still a concern if not monitored. It is possibly better not to get the soil back at all, but there needs to be a way of containing this soil, especially if graders are also grading ware potatoes at the same facility.

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We discussed the use of cover crops, or potentially biofumigants and trap crops, however the way that crop rotation in Scotland works doesn't easily allow for this. Potato crops are often harvested in September or October and the crop that immediately follows this tends to be winter wheat which is sown immediately after the potato crop is harvested. Winter wheat is therefore the most likely crop in which ground keepers are seen. The rotation does not have a gap in which to plant nematicidal crops such as mustards and those that are planted will not have time to establish. *Solanum sisymbriifolium* does not establish well enough in Scotland to work as a trap crop, or certainly not where there would be a gap in cultivation to allow them to work as a trap crop as it really needs warmer climate. Very few farmers would be willing to lose a summer cash crop to replace it with a biofumigant, unless subsidies were provided to do this.

Greater communication is required about the risks of PCN, what the current situation is, farmers who did not have PCN or who had only lost a few fields to PCN don't seem to understand that there is a risk that their land might become infested. A couple of the farmers that we interviewed knew almost nothing about PCN, very few were fully aware of the biology of PCN, how they multiply, how they spread and the damage they can cause. Growers were predominantly concerned about whether or not they could use the land for seed because of PCN but did not always understand the potential yield losses or long term damage PCN could cause if left unchecked. Those who have PCN on their land seem to think that if they get a negative de-recording that the cysts have all gone, there needs to be education about sampling and that where a famer has a patchwork pattern of results that there is a low level infestation across the farm.

There are a lot of issues of particular concern in Scotland e.g. the lack of ability to cultivate processing ware varieties, lack of ability to use bio-fumigants and trap crops, which other countries are using in order to control PCN populations.

Another aspect that became apparent was the lack of interaction between farmers, each one had their own management strategy and there was a feeling that they did not wish to share information with their neighbours. As PCN and the management is more important than just at the farm level it is important that management strategies are at a more strategic level and have buy-in from growers and producers.

5.3 Information provided to growers

Actions following on from this report include providing growers with further information about PCN are and what their test results mean. This could be issued by SASA and other regulatory bodies. Examples of advice that could be given to growers under different situations are given below:

Initial clear

Because you had a clear result you are free to plant whichever seed you wish, however there are measures you can take which will help keep the land below a detectable level of PCN and therefore in seed production. Consider what is on nearby land, if all the land you use is free from PCN this is a good sign. If some of the land you use has tested positive for PCN then there are PCN in the area and you need to be particularly careful not to spread these around. Consider the varieties that you plant. One of the most effective ways of controlling the PCN population is to use resistant varieties, further information about resistant varieties can be found at the European potato database http://www.europotato.org/ or the Potato Variety Database. Long rotations also help to control PCN as each year 20-30% of the eggs contained within the cyst will die off. It

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may be worth doing additional testing through a commercial laboratory to find out more about the PCN situation on your land. Always only use certified seed.

Fail
This land cannot be used for the production of seed potatoes. Options are to take the land completely out of potato production, the population decreases annually and it is possible that it will reach below detectable levels when the land is next eligible and thereby keeping the land in seed production. Alternatively ware can be grown under a control programme. The purpose of the control programme is to allow ware to be produced in a way that reduces the population of PCN in the long term. The most effective way of bringing land back in to seed production is the use of resistant varieties, more information about these can be found at http://www.europotato.org/ or the Potato Variety Database. It is advisable to avoid any movement of soil from the area of land that has failed to the rest of your farm, consider how farm machinery is used. You may wish to carry out your own testing with a commercial laboratory in order to better determine the extent of the outbreak on your land.

De-recording clear
If there is a historical recording of PCN on the land but it has now passed this means that the PCN levels in the land are now below detectable levels, which is good news. Each year some of the eggs inside the cysts will hatch out meaning that the population will decrease year on year and the population in this field is below detectable levels. Please be aware however that the population of cysts on the land will be several million before they are likely to be detected. The fact that the land has previously had PCN, and because cysts can survive for up to 40 years, this suggests that a low level infestation may remain in the field. In order to keep the land in seed production for the foreseeable future it would be wise to consider the use of resistant varieties to the species of PCN you have present. If you are unsure of the level of resistance that your chosen cultivar has please check it at http://www.europotato.org/ or the Potato Variety Database. You should also be very conscious of biosecurity to ensure that if there are PCN in the area these are not spread around.

5.4 Biosecurity recommendations

Biosecurity is defined as a range of preventative measured designed to reduce the risk of spread of an infectious disease or quarantine organism or pest. There are many ways to help prevent land from becoming infested in the first place and minimising the spread of PCN once an infestation on the farm has occurred.

Preventing the initial infestation
- Only use certified seed as that way you can be sure that the land it was grown on was found to be free from PCN prior to planting.
- It is worthwhile regularly testing your own land for PCN, if all your land has been tested as free from PCN, the risk of spread within the farm is minimised.
- If PCN are found, then the PCN in those fields should be actively managed
- If some fields have PCN but others do not, you must be very careful to avoid spreading PCN.
- PCN is spread predominantly with soil; the most important thing is therefore to prevent the movement of soil around the farm.
- Clean all machinery between fields to remove any soil, particular attention should be given between fields which are known to be infested and those thought to be clean

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- It is always important to ensure that any contractors or renters of the land use only clean machinery in order not to inoculate your land.
- Animals (particularly those with hoofs that can accumulate soil) should not be walked between land which has PCN and land which is free from PCN.
- If irrigating land, ensure that the run off from an infested field does not go through a clean field
- Shoes should be brushed to remove all soil, alternatively different shoes should be worn in PCN infested and in clear land.
- Any plants for planting elsewhere must not be grown in infested land. Plants for planting should only be grown on PCN free land, or transplanted free from soil.
- Ensure boxes transporting potatoes are clean and covered.
- Any waste soil should be disposed of in a manner to prevent the spread of PCN (full guidance on preventing the spread of pathogens can be found in the code of practice for the management of agricultural and horticultural waste http://webarchive.nationalarchives.gov.uk/20141216180052/http:/www.fera.defra.gov.uk/plants/publications/documents/copManagementWaste.pdf).

Managing a known infestation

- Find out which species of PCN are present on your land. This is important because varieties with resistance to PCN usually vary in the degree of resistance they exhibit to the two species
- Where possible avoid planting any land with a known low level infestation of PCN with a susceptible ware crop – susceptible ware crops will greatly increase the PCN population of the field and create a far greater risk of spread to surrounding land.
- Nematicides can protect yield but do not prevent PCN increasing when used in conjunction with susceptible varieties.
- Using a ware crop with high resistance to the species present in a field will reduce the population of PCN and make it less likely to be spread.
- Susceptible ware crops should not be planted in infested units adjacent to seed crops, this could render the field unsuitable for future seed production and risk spreading PCN to the seed crop
- Always thoroughly clean down machinery after leaving a field with a known PCN infestation, ensuring that all soil is removed.
- If you have a positive PCN test result, it may be useful to test the land more intensively to find out if there are any hotspots.
- Where hotspots are present cultivation through these will spread cysts so, if possible, should be minimised
- You may wish to consider sectioning off fields to avoid cultivation through PCN hotspots in to clean land.
- Do not transplant plants or soil from infested areas to clean areas
- Groundkeeper potatoes must be removed as these will act as further hosts for PCN, amplifying existing infestations. Groundkeepers of varieties with resistance to PCN may select for more virulent PCN populations capable of overcoming the resistance.

Good hygiene and minimising the spread of PCN will have the added benefit of preventing the spread of a range of soil-borne diseases and is fundamental for sustainable management programmes.

5.5 PCN management

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Along with the previous recommendations to growers, flow charts showing the best management options particularly for ware growers have also been created (figures 23 and 24).

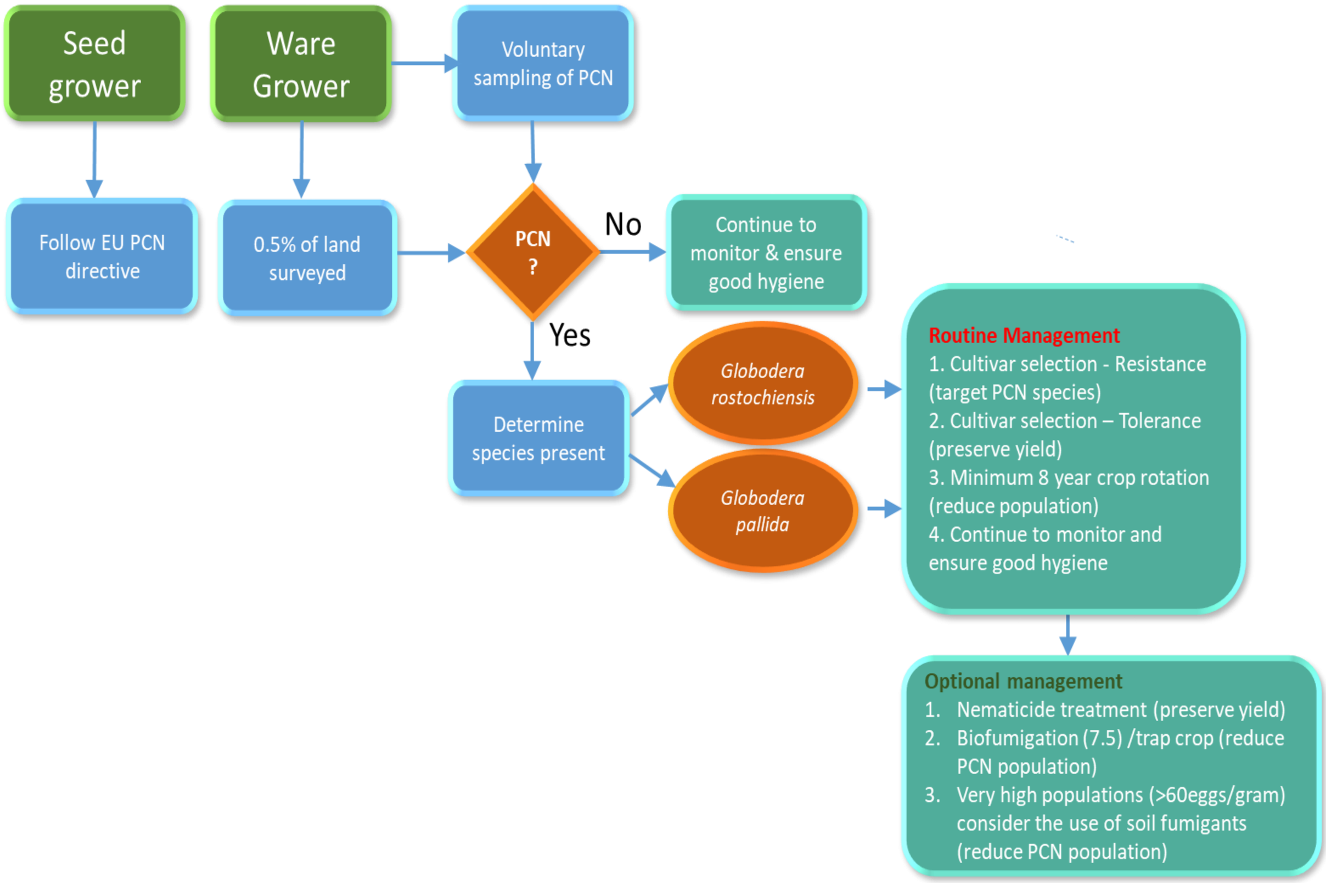

Figure 23: Management recommendations for potato growers

Seed growers must adhere to the EU directive, that is, where PCN are found in a pre-crop soil test the land is recorded and seed potatoes cannot be grown. Ware can be grown under a control programme and these are currently advised and approved by area office managers using the principles outlined in figure 43 and the AHDB potatoes PCN calculator https://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator . Figure 44 on the next page shows what will happen to PCN populations following the cultivation of varieties based on their resistance and tolerance characteristics.

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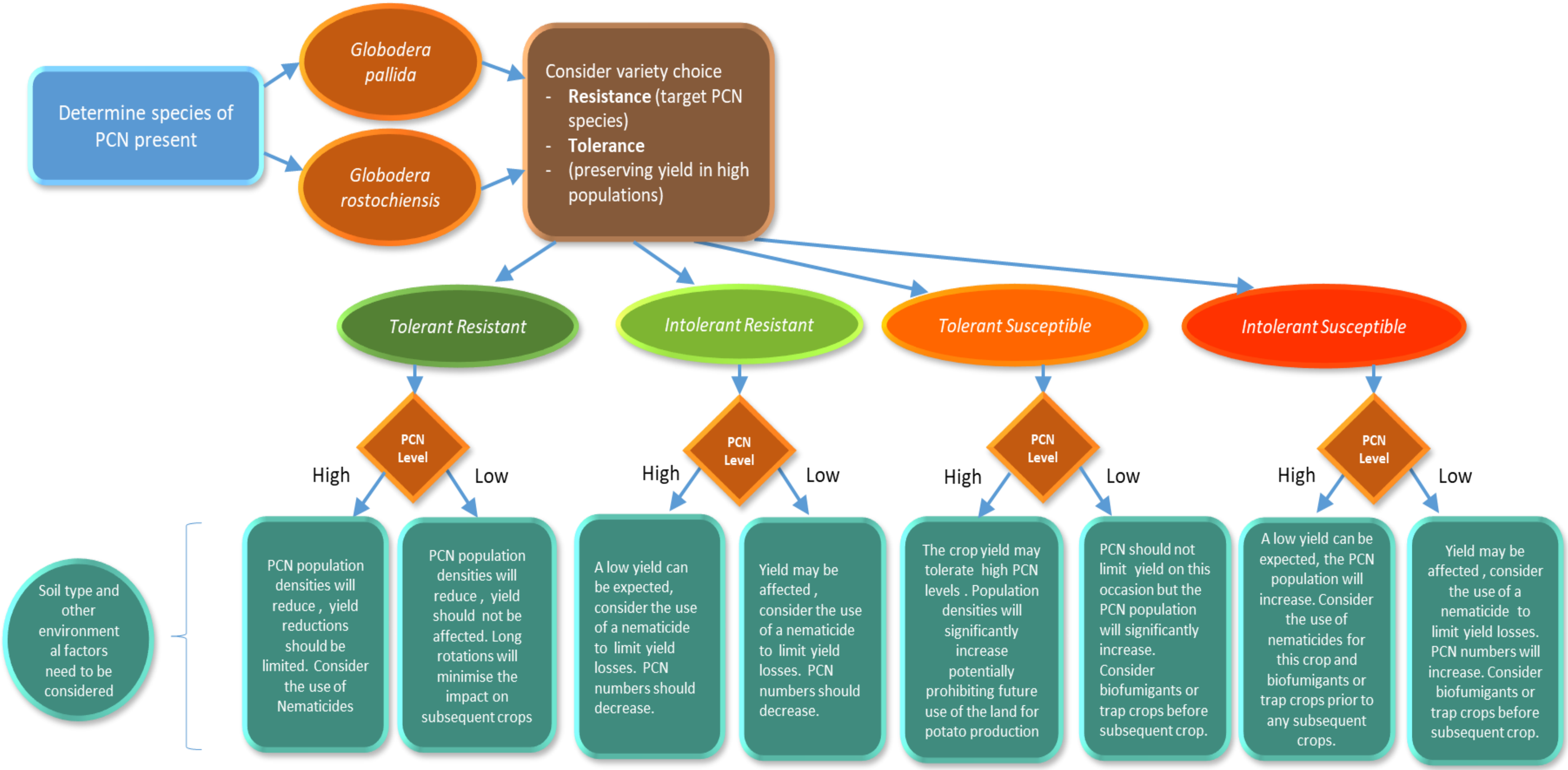

Figure 24: Outcomes of planting dependant on the characteristics of the cultivar planted and the initial PCN population.

One of the questions that the fellow was tasked with answering was "Is it possible to achieve sustainable control under the 2007 EU PCN directive?" The short answer to this question is no, to achieve sustainable control of PCN more needs to be done than simply adhering to the directive. While the incidence of *G. rostochiensis* has remained relatively static since the implementation of the legislation, *G. pallida* has continued to rise. It is currently not known what effect the control programmes are having in managing PCN in infested land. Often when farmers have positive test results the land is lost to ware, which is making the situation worse. PCN in ware land is not regularly monitored and the surveys suggest the situation is far worse in ware than in seed land. There seems to be relatively little advantage in growing seed over ware and there are almost no *G. pallida* resistant varieties that can be grown for ware. The long term future of the potato industry in the North of Britain is therefore in a precarious situation with regards to PCN. The varieties that can be grown for ware are generally table varieties and the table sector is decreasing. At the same time the land available for seed is decreasing due to PCN, turning more growers away from seed and into ware production. Many of the alternative control strategies for PCN are not suitable for the climate and farming practices in the North of Britain. A whole industry strategic approach to the sustainable management of PCN is therefore required.

6. GAP ANALYSIS

- Obtaining more data on representative field distributions: The field data on PCN distributions chosen for the soil sampling study were not selected using any unbiased criteria, we simply obtained data from as many sources and as many fields as were made available to us. As several fields represent trial sites, it is likely that any bias will be towards more highly infected fields. Therefore, it is uncertain how representative of the British PCN situation the data used for this analysis is. To address this further, data could be obtained from more sites and potentially more field work could be carried out to fill in the gaps. These data would allow further analysis of the relationship between heterogeneity and density of PCN populations.

- Obtaining geostatistical data from British PCN field distributions: The lack of available intensively mapped, geostatistical information from a range of fields prevents any conclusions being drawn on the precise way to divide fields for sampling, and from assessing the implications of some sampling schemes in common use, e.g. a ring of samples taken from around a GPS located quad bike/tractor. Such intensively mapped data would permit more detailed exploration of models for the spatial distribution of PCN. They would allow more detailed evaluation of the general applicability of the distribution described by the Been & Schomaker models. However, the collection of such detailed information from a representative range of fields is an extensive and expensive undertaking which may or may not provide sufficiently consistent information from which to reach clear conclusions on sampling. Furthermore, we feel it is unlikely that any such conclusions would lead to any significant differences in our recommendations for sampling to detect PCN.

- Additional information may enhance the sampling procedure and the estimates coming from it. One possibility is to direct or supplement the tests using related covariates, perhaps using satellites or drones (Wyse-Pester et al., 2002; Anon.,1997 Anon., 2015).The use of drones and satellite imagery could be used to provide additional information based on images of the damage caused by PCN to ware potato crops. These could be valuable in mapping the shape of patches with high population levels. The downside of this approach is that low level infestations are unlikely to produce visible symptoms and any damage seen is likely to be causing yield loss and has the potential to leave even more damaging populations of PCN. As potato crops are affected by a wide range of pests and diseases, it should also not be automatically assumed that the damage has been caused by cyst nematodes. Therefore a limited amount of confirmatory soil sampling from affected areas should be carried out to support any such remote sensing approaches.

- Cost/benefit analysis of PCN testing: A financial analysis of different testing options would complement this project. Factors that should be considered is cost of time to walk different sampling patterns across a field, different laboratories will charge different amounts for resting soil samples.

- Subsampling: Further work could be done to define a protocol for the effective subsampling of soil samples for PCN detection or quantification. This might involve seeding samples in order to determine how samples can be effectively mixed and subsampled taking account of factors such as soil type and soil moisture. One issue that should be addressed is the potential for PCN cysts to aggregate/separate out as a dry soil sample is mixed. Along with this, samples could be taken from fields known to harbour moderate PCN infestations to determine how within a soil sample the cysts are aggregated and how to ensure uniformity between subsamples. Within this there is also scope to investigate the relationship between eggs per gram, eggs per cyst and cysts per kilogram to determine the risks of subsampling where cysts are highly viable. Obtaining truly representative subsamples is critical to the effectiveness of the sampling guidance outlined here and the development of a standard protocol for laboratories to use for subsampling could be invaluable.

- Estimation of the likely aggregation of eggs within cysts: As this is a key factor in determining the recommended volume for any laboratory subsampling, a method to estimate the likely numbers of eggs/cyst would be valuable. This is likely to depend upon decline rates and how they likely to vary between factors such as species and soil type.

- The aim of control programs is to allow sustainable production of ware potatoes by reducing the population of PCN in a field. How effective these control programmes are in achieving their aim are yet to be assessed. Control programmes for ware crops are based on the AHDB PCN calculator. The outcomes of the PCN calculator are also heavily dependent upon soil type, but this information has yet to be included in SASA's Seed Potato Users Database (SPUDS). Requirements of the control programme often include the use of resistant varieties where available, nematicide application and a stipulation of the rotation length that should be adhered to along with groundkeeper control requirements. Alternative control methods such as the use of trap crops or

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biofumigants are also considered and real field examples of how effective these controls are required.

- It would be valuable to have an insights into decline rates in different soil types in Scottish climatic conditions. It is believed that populations may decline initially fairly rapidly with a tailing off of decline rates in the field and it would be valuable to test this assertion. A survey of pathotypes and mitotypes will allow us to determine if different variants of *G. pallida* do better under different conditions (soil type, climate, geographical location) and also whether different control options vary in effectiveness depending on the PCN population present.

- It is worth doing economic impact assessment of PCN so that growers can understand financial and yield risks to crop long term if don't manage the issue now. It would be helpful to put together maps and graphs to show how population increasing in a way that is easy to understand. To look at future scenario's using a change nothing approach to show where this will lead us in 10, 20 50 years etc. in order to really raise awareness.

- More work could be done with alternative control strategies and how to make them suitable for Scottish or British conditions. Can new trap crops be developed that are more suited to cooler climates or can biofumigants be developed that work better specifically in these conditions? Is there any more that can be done with hatching factors to develop a useable product to initiate hatching? There could be more potential uses for fungal and bacterial pathogens, but again its getting these to be effective in field rather than just laboratory conditions.

- It would be valuable to make a risk assessment with regards to initial infestations of PCN and other nematodes which may be of concern to plant health. There have been anecdotal reports of garden waste material being used post composting on agricultural land. This waste material may contain peelings from potatoes which may not have a UK origin or even soil from plant pots and therefore could present a risk depending on how effective the composting procedure is. A thorough assessment should be made of the risk, is this material used on agricultural land and if so where? A study should be made to assess the survival of nematodes through the recycling and composting procedure used to ensure this is not a potential route of infection.

- Another related issue may be what happens to PCN when they travel through the guts of an animal, this has been raised a couple of times recently. It has been suggested that PCN can remain viable after passing through the guts of an animal. Ware crops often go as cattle food if there is no market for them if slurry from these animals then go on to fertilise land in which seed potatoes are grown this could be an initial source of infection for the crops. It would be useful to find out where these stock potatoes are sourced from as well, while in Scotland it is believed these are all sourced locally this may not be the case for all of GB and could be a potential route into the country for potato pathogens.

- One area where further developments could be made is in the use of drones. This could for mapping outbreaks and then relating them to sampling data in

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order to establish if drones could be used more effectively for determining levels of PCN outbreaks in a field. Would it be possible to use drones to show where outbreaks in a field occur? Longer term could they be developed to take soil samples?

- With *Globodera ellintonia* being discovered in Oregon in 2008 (Handoo *et al*., 2012) it would be useful to learn more about the impact they could have on common commercial potato cultivars. It would useful to set up some resistance trials with some potatoes that are commonly grown and those with *Globodera pallida* or *Globodera rostochiensis* resistance and also to assess the impact they have on yield.

- *Meloidogyne* spp. (Root Knot Nematodes) are considered to be the most economically important genus of nematode worldwide. Their current impact in Great Britain has, to date, been restricted to cases on sports turf, where damage has been linked to *M. minor* or *M. naasi*. The discovery of *M. fallax* and *M. chitwoodi* in Europe in the late 20th century led to both species acquiring quarantine status. Shortly afterwards, DNA diagnostics on preserved specimens from 60 or so years earlier found that symptoms previously attributed to *M. hapla* were actually caused by one of these 'new' species. With the species now known to have been present in some major seed potato producing areas within the EC for the last 80 years, it is inevitable that these pests will already have been transmitted to many parts of the world. Impacts on areas where potatoes are grown in warm countries with light sandy soils supported by pivot irrigation have been particularly severe. The scarcity of imports of potatoes into Scotland means that we should have been far less exposed to this risk, however we should raise the profile of the plant health risks posed by *Meloidogyne* to Britain, assess risk points of entry and perhaps carry out survey work to ensure the country remains free from this damaging pest.

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<u>Web-links</u>

https://www.hutton.ac.uk/research/groups/cell-and-molecular-sciences/potato-cyst-nematodes

http://randd.defra.gov.uk/Default.aspx?Module=More&Location=None&ProjectID=9913

https://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator

http://webarchive.nationalarchives.gov.uk/20141216180052/http:/www.fera.defra.gov.uk/plants/publications/documents/copManagementWaste.pdf

https://www.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/agritopics/Pots

http://varieties .ahdb.org.uk

http://www.europotato.org/

https:ahdb.org.uk/pcn-grower-guide

https://potatoes.ahdb.org.uk/publications/pcn-sampling-and-laboratory-guide

8. ACKNOWLEDGEMENTS

Thank you to everyone that has contributed to this project in any way. I must thank Jon Pickup for all of the knowledge he has passed on to me over the past 3 years. Thanks also to Yvonne Cole and Alex Reid for helping me with projects, allowing staff time to work with me and for their specialist advice. To Yvonne Cole and Mairi Carnegie for co-running the workshops for the laboratories. Thank you to any SASA staff and Inspectorate staff that have helped throughout this project.

I am grateful to all of the input I have had from other nematologists across the UK, from JHI, FERA, Harper Adams, Leeds University etc. and to Nematologists I have worked with across Europe and at an International level, there are too many to list them all. To John Keer (Richard Austin) Nick Winmill, Eric Anderson (Scottish Agronomy), Andy Barker and Emma Keltcher for providing data for the soil sampling project.

All Growers who agreed to be interviewed this project, and for those growers who provided feedback about PCN management to me at Potatoes in Practice and the seed potato growers roadshow. To producers that we interviewed including McCain,

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Greenvale, Irish Potato Marketing, A.J.Allan, Grampian Growers. To Potato breeders – Cygnet and JHI ltd for talking me through the process and for discussions about breeding in resistance.

To Irene Wilderboer for her input to this work, who brought enthusiasm to the project and imparting information about the potato industry in the Netherlands. To Adrian Roberts from BIOSS for all his statistical input. AHDB potatoes – for giving me this opportunity by funding the work and particularly to Sue Cowgill who provided support and guidance throughout the project.

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