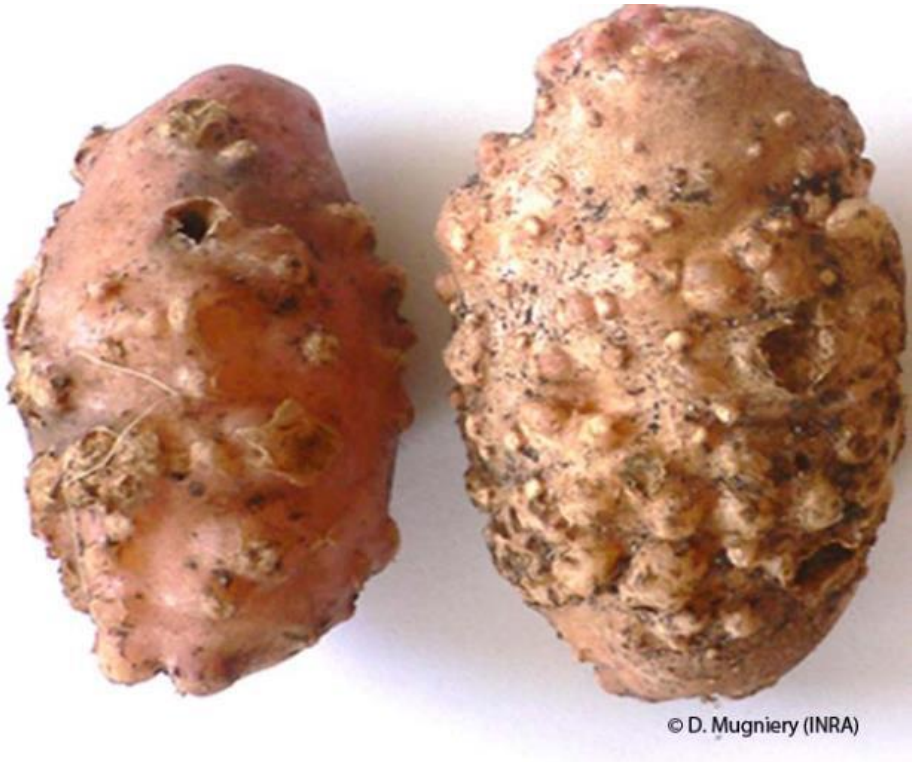
Assessment and mitigation of the threat posed by root-knot nematodes to potato production in the UK

Project Final Report



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Content

1 Executive summary

Root-knot nematodes (*Meloidogyne* spp. - RKN) are the most economically important plant parasitic nematodes on a global basis. Many species can feed on an extremely broad range of plant hosts. Regulated RKN species have not been identified in Scottish soils and the most damaging species tend to establish in warm climates, so they have limited ability to survive the harsh winter conditions found in Scotland. However, recent studies on emerging pathogen threats indicate that root-knot nematodes are among the fastest spreading pathogens in response to climate change. This project sought to address two main aims. First, we aimed to better understand the potential threat posed by a range of root-knot nematode species to future Scottish potato production. Second, we sought to determine the prevalence of resistance against one root-knot nematode species, *Meloidogyne incognita*, in a germplasm collection hosted at the James Hutton Institute. Key findings can be summarised as:

- On the basis of distribution records of RKN within Europe, *Meloidogyne chitwoodi* and *M. fallax*, were the two species of most concern and which have the potential to cause severe damage to the Scottish potato crop and other crops commonly used in the rotation.
- Border controls currently in place have been sufficient to prevent introduction of either species to date.
- *Meloidogyne chitwoodi* and *M. fallax* have not been found in Scottish soils, but in order to maintain vigilance, raising awareness of symptoms of root-knot nematode infection and ensuring training is kept up to date, particularly among those undertaking potato tuber inspections, would potentially help in early awareness of the presence of root-knot nematodes.
- Analysis of climate modelling data suggests that even under the most extreme predicted climate change scenarios, conditions in Scotland are unlikely to allow thermophilic species of RKN nematodes to establish, except under protected cropping conditions.
- Climate change may exacerbate the damage caused by endogenous species of RKN due to the impact of nematode infection on the ability of plants to take up water.
- Reductions in the availability and use of nematicides elsewhere in Scottish rotations may increase incidental damage from established RKN species to carrots, parsnips and potato (*M. hapla*) or cereals (*M. naasi*).
- The effectiveness of cover crops against RKN under UK conditions is not well understood. If available nematicides continue to be withdrawn it will be essential to provide growers with information about alternative control strategies, including cover crops.
- Caution is required when using cover crops as some widely grown species may well be hosts for some RKN species, or for free living nematodes.
- Although some potato varieties with partial resistance are available, there are currently not sufficient options available for growers should RKN species become a major problem in Scotland.
- Risks of RKN spread identified in a review of literature include spread and introduction from soil movements, poorly composted waste, from the movement of sand (*M. minor*).
- Best practice advice should focus on good hygiene measures, particularly with regards to soil movements. The use of resistant varieties remains a useful tool where this is available.

- The Commonwealth Potato collection remains an underexploited source of potential resistance against RKN.
- Twenty-five lines were recorded historically as having some resistance to *Meloidogyne incognita*.
- Experimental screening undertaken as part of this project confirmed that resistance against *M. incognita* was present in at least two species present in the Commonwealth Potato Collection.
- Future work (funded externally from PHC) will examine the genetics of these new resistance sources.
- Although regulated RKN species have not currently been identified through official inspections of produce grown in Scottish soils, no detailed assessment for non-regulated RKN species are undertaken, so this remains a knowledge gap.
- The use of cover crops and wildflower strips may increase as part of future policy or as part of the uptake of more regenerative practices and their impact on RKN numbers and distribution is another knowledge gap identified.

2 Screening selected accessions from the Commonwealth Potato Collection for resistance against the root-knot nematode *Meloidogyne incognita*

2.1 Introduction

Root-knot nematodes (RKN, *Meloidogyne spp.*) are the most economically damaging plant-parasitic nematodes worldwide and consume up to 10% of all global agricultural outputs (Jones *et al.*, 2013). Some species have extremely broad host ranges, parasitising almost all vascular plants, making management extremely challenging. RKN feed and reproduce within the roots of host plants causing gall, or root-knot, formation which limits nutrient uptake, reducing plant growth and severely impacting crop yield and quality. Above ground symptoms of infection are non-specific and include suppressed shoot growth, chlorosis in foliage and wilting. *Meloidogyne incognita* is one of the most common RKN species globally. As a thermophilic species *M. incognita* will only hatch in warmer conditions, however, it is one of the fastest spreading pathogens in response to current climate change conditions (Bebber *et al.,* 2013).

The Commonwealth Potato Collection (CPC) (https://ics.hutton.ac.uk/germinate-cpc/#/home) represents a unique resource for identification of new resistance genes. Multiple sources of resistance against potato pathogens including potato cyst nematodes (PCN) and late blight (*Phytopthora infestans*) have been identified and incorporated into breeding programmes (*e.g.* Bradshaw & Ramsay, 2005). However, this remains an underexploited source of potential resistance against RKN species. This project aimed to phenotype multiple CPC accessions (individual plant samples deposited and maintained within the collection) for resistance against *M. incognita* to identify a resource for future-proofing the UK potato sector. The Sturgeon Bay catalogue has published a list of 'Accessions with reputed resistance to root-knot nematodes'. PI numbers from this list were cross referenced with the US National Plant Germplasm System (available: https://npgsweb.ars-grin.gov/gringlobal/search) to identify accessions that were duplicated in the CPC. Original collecting numbers were consequently checked against collecting information as occasionally the Sturgeon Bay catalogue has renamed species. Taxonomic keys and original collecting information were verified by J. G. Hawkes on a previous visit to the CPC. This cross-referencing allowed production of a series of *Solanum* spp. with accessions held within the CPC that potentially contains RKN resistance. This report summarises phenotypic screening of these accessions against *M. incognita*.

2.2 Methods

2.2.1 Commonwealth potato collection accessions

All the varieties available to be tested (summarised in Table 1), were grown in greenhouse conditions (16h day length at 20 °C, 8h 16 °C at night, ± 4 °C). However, being wild varieties with different growing requirements, 5 accessions (CPC7636, CPC7640, CPC7654, CPC7791, CPC3745) were unable to grow under these generalised conditions.

In J. G. Hawkes' book 'The Potato, Evolution, Biodiversity & Genetic Resources' table 7.1 (1990) suggests that approximately 16.3% of native Andean cultivars that were tested contain resistance to *M. incognita*. Accessions in the CPC are rejuvenated on a rotating basis to produce fresh seed stock. Opportunistically, the current round of accessions being rejuvenated were also screened for *M. incognita* resistance and summarised in Table 1. Additionally, spare plants from germinated *S. iopetalum* (IOP2922) being grown for display purposes were screened for RKN resistance.

Table 1 - Commonwealth potato collection varieties screened for Meloidogyne incognita resistance. SB = Sturgeon Bay. More information about accessions is available at: https://ics.hutton.ac.uk/germinate-cpc/#/home

CPC Accession	Genus	Species	Origin	In SB catalogue?
CPC 2683	*Solanum*	*agrimonifolium*	Guatemala	Y
CPC 3532	*Solanum*	*canasense*	Peru	Y
CPC 3057	*Solanum*	*chacoense*	Argentina	Y
CPC 2610	*Solanum*	*fendleri*	USA	Y
CPC 3777	*Solanum*	*oplocense*	Argentina	Y
CPC 7047	*Solanum*	*guerreroense*	Mexico	Y
CPC 3208	*Solanum*	*hjertingii*	Mexico	Y
CPC 7049	*Solanum*	*hougasii*	Mexico	Y
CPC 7166	*Solanum*	*jamesii*	USA	Y
CPC 7786	*Solanum*	*polyadenium*	/	Y
CPC 2308	*Solanum*	*polytrichon*	Mexico	Y
CPC 7107	*Solanum*	*sogarandinum*	Peru	Y
CPC 3744	*Solanum*	*spegazzinii*	Argentina	Y
CPC 3780	*Solanum*	*spegazzinii*	Argentina	Y
CPC 7796	*Solanum*	*verrucosum*	/	Y
CPC 7781	*Solanum*	*vernei*	Argentina	Y
CPC 7797	*Solanum*	*vernei*	/	Y
CPC 2618	*Solanum*	*stoloniferum*	Mexico	N
CPC 3777	*Solanum*	*oplocense*	Argentina	N
CPC 54	*Solanum*	*verrucosum*	Mexico	N
CPC 5696	*Solanum*	*acaule*	/	N
CPC 2727	*Solanum*	*Microdontum (subsp. gigantophyllum)*	Argentina	N
CPC 2713	*Solanum*	*stoloniferum*	Mexico	N
CPC 7811	*Solanum*	*fraxinifolium*	Costa Rica	N
CPC 2533	*Solanum*	*sparsipilum*	/	N
CPC 7812	*Solanum*	*huancabambense*	Peru	N
N/A	*Solanum*	*iopetalum*	/	N
CPC 6065	*Solanum*	*kurtzianum*	/	N

2.2.2 *Root-knot nematodes*

Meloidogyne incognita was grown on tomato plants (cultivar Moneymaker) under greenhouse conditions (16h day length at 25 °C, 8h 20 °C at night, ± 4 °C). Nematodes were allowed to infect host plants for 12 weeks after which the roots were removed, cut into smaller pieces and mixed with 50:50 sand:loam which a new tomato host plant was planted into.

2.2.3 *Screening in Rootrainers*

CPC accessions were grown under greenhouse conditions (16h day length at 20 °C, 8h at 16 °C at night, ± 4 °C) in small seedling pots. When sufficiently sized (approximately 2-3 weeks old, depending on species) plants were transferred to a warmer cubicle for RKN infection (16h day length at 25 °C, 8h at 20 °C at night, ± 4 °C). 50:50 sand:loam mixed with RKN infected tomato roots was used to ¾ fill 4-cell Rootrainers (Haxnicks). CPC plants were individually planted per cell on a rootrainer and grown for 12 weeks. Where possible a minimum of 4 plants per accession were screened against nematodes. Having been grown from true seed, variation in resistance within accessions was expected. After 12 weeks, Rootrainers were opened and signs of nematode infection were scored either showing no infection, light galling, heavy galling, or dead plants possibly due to the different growth requirements of different accessions (Figure 1). Each of the 4 sides of the root system was checked for RKN-induced root galling.

Figure 1 - Meloidogyne incognita infection assay scoring in Rootrainers. Signs of heavy galling have been marked in red circles.

2.2.4 Cuttings and rescreening

Cuttings were taken from donor plants where RKN resistance was identified. Stems were cut approximately 2 cm beneath a node and dipped into rooting gel (Clonex). Gel covered cuttings were placed into a pre-soaked peat-plug (Jiffy-7) which was placed in a tray and covered to keep the plug damp. Trays of plugs were stored in greenhouses (16h day length at 20 °C, 8h 16 °C at night, ± 4 °C) and watered regularly to prevent drying out. When roots were visibly growing out the side of the peat-plugs they were transferred to 4-inch pots with standard potting mix (peat:wood fibre 70:30, limestone, Osmocote start 12+14+24, Osmocote standard 16+9+12, H_2GRO wetting agent, perlite). Once daughter plants had sufficiently grown several further cuttings were taken. This time, once roots were visible outside the plug it was transferred to fresh RKN infected rootrainers (Haxnicks, deep) for rescreening. Original plants were kept for tuber collection where possible.

2.3 Results and Discussion

Of the 258 plants screened for phenotypic *M. incognita* resistance, over 65% presented with a combination of light or heavy galling (Figure 2).

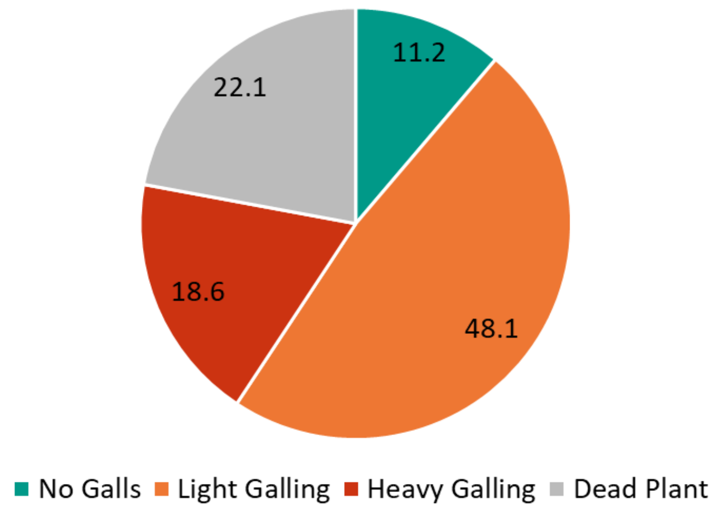

Figure 2 – Phenotypic response of tested commonwealth potato collection accessions in response to M. incognita after first round of screening. Note that dead plants are likely due to unfavourable growing conditions and not a response to nematode infection. Values represent percentage of total plants tested.

When looking at the phenotypic results per accession (Figure 3) it is clear that some species have no *M. incognita* resistance, regardless of whether they were identified in the Sturgeon Bay catalogue as having resistance (e.g. *S. guerreroense*, GRR7047 and *S. chacoense*, CHC3057). Where phenotypic resistance was visible there was noticeably a 50:50 or 1 in 4 segregation between number of resistant plants and number of susceptible plants (e.g. *S. vernei*, VER7781 and *S. fendleri*, FEN2610). As plants were grown from true seed variation in genotype, and so variation in resistance phenotype, was expected.

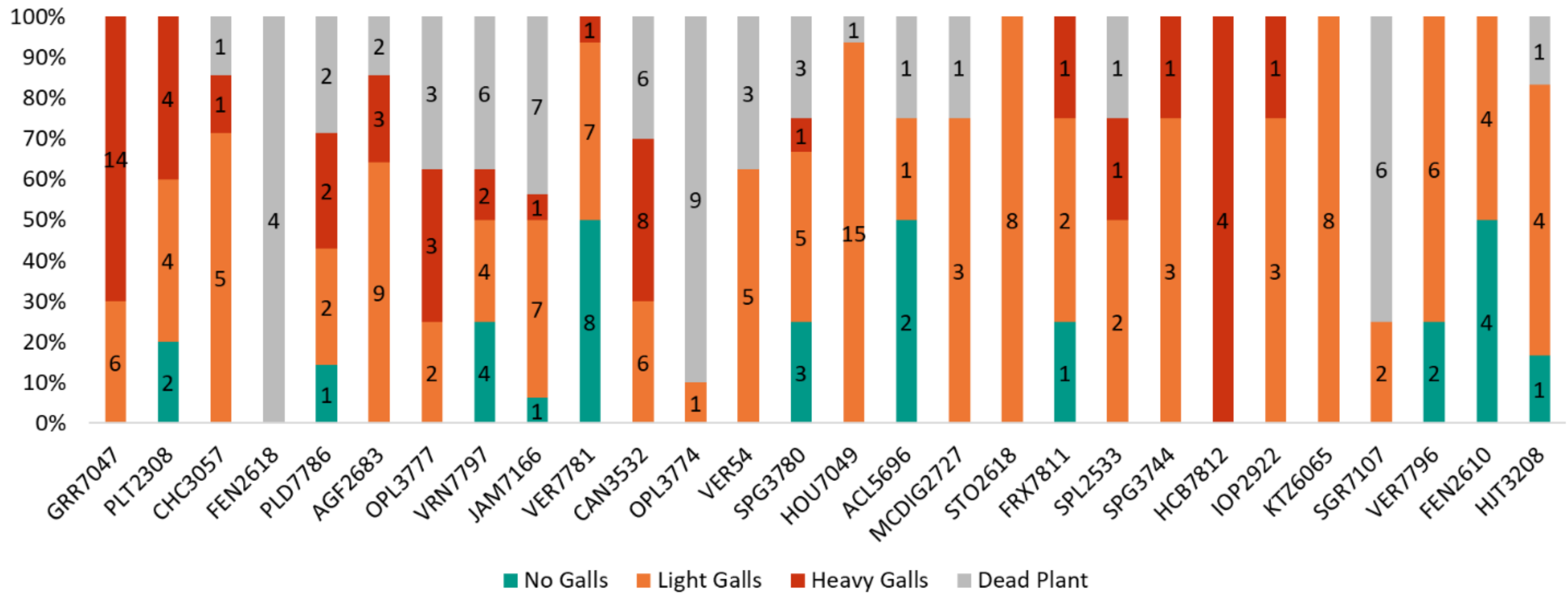

Figure 3 – Phenotypic response of commonwealth potato collection accessions in response to M. incognita per accession tested after first round of screening. Values within bars represents number of plants.

Cuttings were taken from donor plants marked as resistant to *M. incognita* for a second round of screening. No cutting was taken from the single *S. jamseii* plants that was scored as resistant out of 16 plants tested as this was likely an artefact due to a failure of the nematodes to infect in this replicate. Cuttings for VER7781, VER7796, VRN7797 and HJT3208 all died and so were unable to be rescreened. Both ACL5696 and FRX7811 had unusually slow growth habits and rescreening was not possible within this project.

Rescreening of plants previously identified as resistant to RKN infection confirmed the resistant phenotype with 40% of plants being scored as resistant (Figure 4).

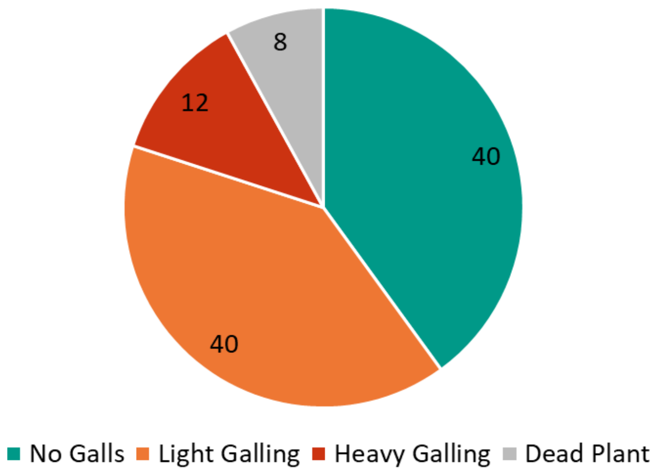

Figure 4 - Phenotypic response of rescreened commonwealth potato collection accessions in response to M. incognita. Note that dead plants are likely due to unfavourable growing conditions and not a response to nematode infection. Values represent percentage of total plants tested.

Phenotyping per accession (Figure 5) shows that some of the original donor plants identified as resistant were false positives. SPG3780, like SPG3744 has no resistance to *M. incognita*. The single PLD7786 plant with no galls in the first round of screening that was selected for rescreening likely had minor galling that was missed.

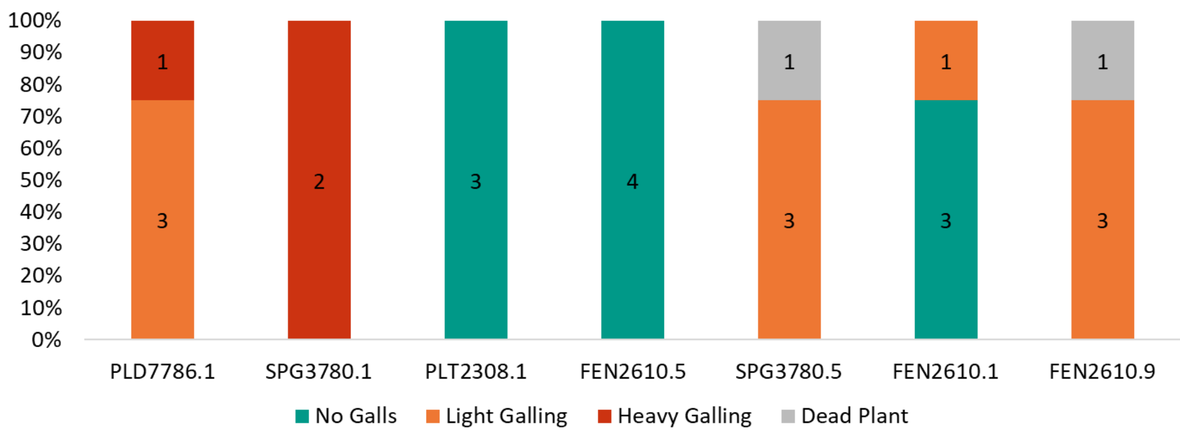

Figure 5 – Phenotypic response of cuttings from commonwealth potato collection accessions previously identified as being resistant to M. incognita. Additional number after the CPC value identifies cutting number. Values within bars represents number of plants.

S. fendleri (FEN2610) and *S. polytrichon* (PLT2308) showed good resistance against *M. incognita*. These species are from the Solanaceous series *Longipedicellata* which are Mexican tetraploids with similar growth habits found in roughly similar conditions. *S. hjertingii and S. stoloniferum* are also part of the *Longipedicellata* series and were tested for resistance during this project (HJT3208 & STO2618). However, no notable resistance was scored for these accessions. Other species within this series that should be screened for RKN resistance include *S. matehualae* and *S. papita*.

Morphologically, *Longipedicellata* species are similar to *S. verrucosum* (*Tuberosa*). In this study VER7796 and VER7781 were both identified as containing some resistance against *M. incognita*, however, cuttings did not survive and resistance could not be confirmed. While no resistance was recorded in VER54, light galling was noted as being very light. *S. verrucosum* accessions should therefore be rescreened in future work. Similarly light galling was noted in HOU7049, MCDIG2727 and KTZ6065 which should also be included in rescreening.

Tubers from resistant FEN2610 and PLT2308 cuttings have been collected and stored for future work. These new potential resistance sources will be further investigated, including mapping and more detailed phenotypical analysis, in a recently awarded PhD studentship.

2.4 References

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3 Desk study: The potential threat to crop production in Scotland from root-knot nematodes (*Meloidogyne* spp.)

3.1 Summary

- An analysis of the potential threat posed to Scottish potato production from root-knot nematodes has been performed, including an investigation as to the potential impacts of climate change on the ability of thermophilic species to establish. Distribution and potential threats have been reviewed.
- Two species, *Meloidogyne chitwoodi* and *M. fallax* have the potential to cause severe damage to the Scottish potato crop and other crops commonly used in the rotation. Restrictions currently in place have been sufficient to prevent introduction of either species to date.
- Analysis of climate modelling data suggests that even under the most extreme climate change scenarios, conditions in Scotland are unlikely to allow thermophilic species of root-knot nematodes to establish, except under protected cropping conditions.
- It is possible that some non-regulated native and established RKN species in the UK are (unintentionally) kept in check by applications of nematicides used to control virus vector nematodes. Increasing restrictions on use of nematicides may mean that some species become problematic on carrots, parsnips and potato (*M. hapla*) or cereals (*M. naasi*).
- Climate change may exacerbate the damage caused by root-knot nematodes due to impaired ability to take up water.
- Tuber samples designed to give a representative picture of Scotland's national production are brought to SASA for ring rot and brown rot testing are visually inspected for RKN. Although these regular official crop and tuber inspections have not identified any positive findings for regulated RKN species in Scotland, no detailed assessment for the presence of other RKN species have been made, so this remains a knowledge gap. Increased awareness of symptoms of RKN infection among growers would be beneficial in understanding current distribution.
- Best practice could include weed management and use of nematicides, but both of these rely on continued availability of active ingredients and on awareness of presence of RKN.

3.2 Introduction to RKN

Root-knot nematodes (*Meloidogyne* spp.) are a relatively small but hugely important polyphagous (feed on a wide range of plant species) group of highly adapted obligate plant parasitic nematodes. Their taxonomy is complex, but 98 species are currently described in the Genus. Root-knot nematodes are distributed across the world and almost every species of higher plant is parasitised by at least one RKN. They are the most economically important group of plant-parasitic nematodes (Jones *et al.*, 2013). Typically, they reproduce and feed within plant roots and induce small to large galls or root-knots (Figure 6) which give these nematodes their common name. Above ground symptoms tend to be nonspecific and are typical of any plant with a damaged or poorly functioning root system (e.g. stunted growth, chlorosis). Plants heavily infested with RKN may show damage on below-ground harvested products such as tubers. All life stages are small and very difficult to see with the naked eye, although adult females and egg masses may be visible on infected roots (below).

Figure 6 - Tomato roots infected (left) or uninfected with the root-knot nematode Meloidogyne incognita. (Picture from Nemapix).

3.3 Life cycle

The root-knot nematode life cycle is summarised in Figure 7. Adult female nematodes protrude from roots and lay eggs into a gelatinous matrix. Eggs are protected from environmental conditions by a protective gelatinous matrix but are unable to survive in this for long periods of time when compared to eggs of cyst nematodes (*Globodera* & *Heterodera*) in cysts. Development to the juvenile stage, and the first moult, occur within the egg. Second stage juveniles (J2) of RKN hatch from the eggs, with hatch being dependent on temperature and soil moisture rather than the presence of root exudates. Many RKN species have extremely broad host ranges and therefore do not need to coordinate their life cycle with the presence of a specific host. J2 invade the host roots, migrate intercellularly through the root, initially towards the root tip before turning back round the casparian strips (an inner cell layer surrounding the vascular bundle) and into the vascular cylinder (von Mende, 1997). The J2 then induce the formation of several giant cells in the plant. These are formed by induction of nuclear division in the absence of cytokinesis (cell division), resulting in large metabolically active multinucleate cells, which provide the food required for the nematode to develop through a series of moults (J3, J4) to the adult stage. Root-knot nematodes have a highly diversified range of reproductive strategies including facultative meiotic parthenogenesis (where females can reproduce asexually or asexually) and obligatory mitotic parthenogenesis (where diploid eggs form without meiosis in the absence of males). These latter strategies permit the rapid build-up of population levels on new hosts.

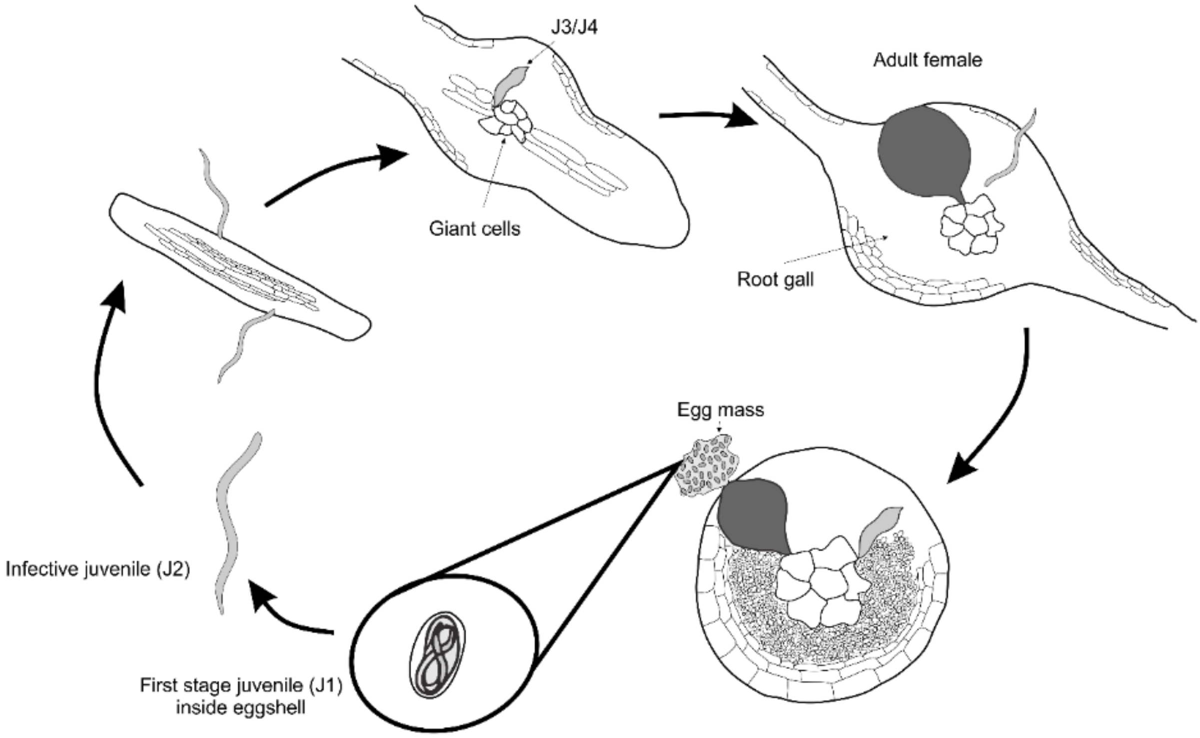

Figure 7 - Generalised life cycle of root-knot nematode. Eggs are laid by the adult female into an egg mass, often visible at the root surface. J2 hatch from eggs, locate and invade host roots and induce the formation of 5-7 giant cells, from which they feed for the duration of the life cycle until the adult female stage is produced.

Root-knot nematodes show remarkable variety in host range. While some species are host-specific, there are many RKN species that have extremely broad host ranges. *Meloidogyne incognita* has been reported as being able to infect almost all species of flowering plants (*e.g.* Eves-van den Akker & Jones, 2018). Four RKN species – *M. incognita, M. arenaria, M. javanica* and *M. hapla* are responsible for the majority of the damage caused by RKN on a worldwide basis. These so called "major species" each have a very broad host range, encompassing monocotyledonous and dicotyledonous plants. However, several other RKN species are becoming increasingly important. For example, *Meloidogyne enterolobii* (previously named *M. mayaguensis*) has a wide host range, is expanding in geographical range and can overcome the widely used *Mi-1* resistance gene deployed in certain varieties tomatoes. In terms of the threat posed by RKN to the UK, *M. chitwoodi* and *M. fallax* are of particular concern as they are temperate and have a broad host range that includes many monocotyledonous and dicotyledenous plants (Moens *et al.,* 2009).

Root-knot nematodes can be divided into two distinct groups based on their temperature requirements. Temperate species (cryophiles) can survive soil temperatures below 5 °C; these include *M. fallax, M. chitwoodi, M. hapla* and *M. minor*. Some of the most damaging species, including *M. incognita, M. arenaria, M. javanica* and *M. enterolobii* are thermophiles and are not able to survive exposure to prolonged periods (variously described as 5 days or 10 days in the literature) where temperatures are below 5 °C. It is this temperature requirement that prevents establishment of these species in a wider range of geographical zones. Notably, an analysis of the spread of pests and diseases in relation to climate change has found that thermophilic RKN species are among the fastest poleward spreading pathogens (Bebber *et al.,* 2013).

3.4 Diagnostics and Sampling

In order to understand the damage potential of any RKN present in a soil it is first necessary to detect, quantify and identify the nematodes that are present. Traditional morphological diagnostics are extremely challenging for root-knot nematodes and the specialist skills needed for this are in increasingly short supply. However, several molecular techniques are widely used for identification of RKN species. Enzyme specific (isozyme) staining of proteins extracted from adult female RKN has been widely used for diagnosis of RKN (Blok & Powers, 2009). A wide range of DNA-based approaches are also available for identification of RKN species (*e.g.* Janssen *et al.,* 2016).

Sampling for RKN can be done by extracting total nematodes from soil samples taken across a field and then seeking to identify whether RKN are present, either using morphometric (visual) or molecular methods. However, this is an expensive and time-consuming process. Extracting nematodes from roots or assessing damage to root systems using root gall indices may also be used as a method for assessing whether RKN are present. Some RKN may cause clear and symptomatic damage to harvested parts of crop plants (Figure 8), allowing the presence of RKN to be confirmed.

Figure 8 - Damage to potato tuber caused by M. chitwoodi (left panel) and to carrot caused by Meloidogyne hapla (right panel). Images by Tom Prior (FERA Science).

3.5 Records of RKN in the UK and Europe

An analysis of published literature and EPPO database records suggests that 25 RKN species have been identified as being present in Europe (Table 2). These include both thermophilic and temperate species.

Table 2: Occurrence of Meloidogyne species in Europe. Based on Wesemael et al. (2011), analysis of EPPO databases (https://gd.eppo.int/) and T. Prior (pers. comm). Background colour indicates temperate (blue) or thermophilic (red) species. Note: In some cases host range may be broader than indicated: for lesser species for which few records are available, this may not have been investigated.

Species	Host range	Occurrence in Europe
M. arenaria	Broad	Widespread
M. javanica	Broad	Widespread
M. exigua	Broad	Greece, Italy
M. incognita	Broad	Widespread
M. enterolobii	Broad	Switzerland
M. lusitanica	Only reported on olive to date	Portugal
M. baetica	Olive	Spain
M. graminicola	Rice, cereals	Italy
M. luci	Broad	Portugal, Italy, Slovenia

Species	Host range	Occurrence in Europe
M. hapla	Broad	Widespread
M. artiellia	Broad	France, Greece, Italy, Spain, UK
M. graminis	Grasses and cereals	Germany, The Netherlands
M. naasi	Grasses and cereals	Widespread
M. ardenensis	Broad	Widespread
M. ethiopica	Broad	Slovenia
M. chitwoodi	Broad	Widespread
M. kralli	Cyperaceae, grasses	Northern Europe, UK
M. hispanica	Broad	France, Portugal, Spain, The Netherlands
M. maritima	Dune grasses	Widespread
M. fallax	Broad	Belgium, France, Germany The Netherlands, Switzerland, UK
M. duytsi	Dune grasses	Western Europe coastal regions
M. mali (=*M. ulmi*)	Broad, mainly reported on elm	Italy, The Netherlands, UK
M. minor	Broad	Widespread
M. dunensis	Sea rocket	Spain
M. sylvestris	Holly, woody shrubs	Spain

A smaller number of species have been identified in the UK, including native species as well as introductions that have established or, in one case, eradicated (Table 3). None of these are thermophilic species. It should be noted that *M. fallax* has not been recorded in Scotland

Table 3: Root-knot nematode species recorded in the UK.

Species	Host range in UK	Notes	Status
M. minor	Broad	Widespread. Damage due to *M. minor* on anything other than amenity turf rare	Native
M. hapla	Broad	Apparently widespread. Reported to cause damage on ornamentals.	Native
M. naasi	Cereals	Damage more apparent under stress conditions	Native
M. artiellia	Broad		Native
M. ardenensis	Woody hosts, Rosacea		Native
M. duytsi	Dune grasses		Native
M. maritima	Dune grasses		Native
M. kralli	Cyperacea		Native
M. fallax	Broad		Present, restricted distribution
M. mali	Elm	Introduced into UK on Dutch Elm Disease resistant trees. Extent of spread unknown	Established
M. chitwoodi	Broad		Detected and eradicated

3.6 *Potential for establishment in Scotland*

The thermophilic species cannot survive temperatures below 5 °C for more than 5 days, although there are reports that some can survive a short exposure to rapid freezing. Although the summer conditions are sufficiently warm for these species to thrive, winter conditions mean that they cannot establish under current climate conditions. We have investigated whether climate change is likely to lead to the types of conditions that might allow thermophilic species of RKN to survive and thus establish in Scotland. This analysis was carried out using a modified version of the climate data visualisation tool established at the James Hutton Institute (Udugbezi *et al.,* 2022). This analysis suggests that even under the most extreme projected climate change scenarios there will still be periods of more than 5 days where temperatures are below 5 °C (Figure 9), making it very unlikely that thermophilic species will be able to establish in Scotland.

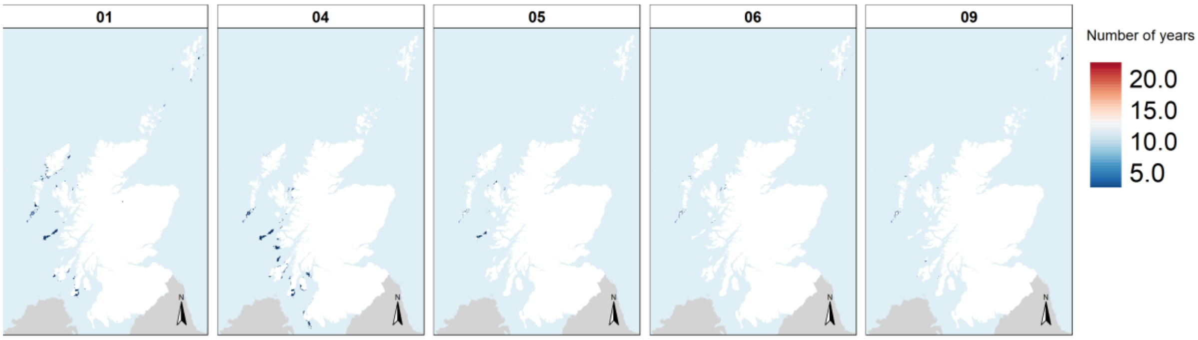

Figure 9 - Climate modelling indicating areas (colour) in which winters where no run of 5 days where the temperature is below 5 °C are likely to occur. Shading indicates number of years where these conditions are likely to occur. Note that coastal areas are prone to false positives due to water temperature impacting temperatures inland.

By contrast, each of the temperate species (Table 2) are very likely to survive if introduced into Scotland. Given that many are widespread in Europe, there is a clear risk of introduction through movement of infected root, soil and tuber materials. Risks of introductions from further afield are mitigated in some cases due to bans on import into Europe and the UK of *Solanum* species. The overall risk of introduction can probably be considered as lower than for potato cyst nematodes as RKN species do not generally have a robust survival stage in the way that potato cyst nematodes (PCN) do.

3.7 *Potential threats to crop production in Scotland*

Given the temperature data presented above, it is extremely unlikely that thermophilic species will establish and pose a threat to crop production in Scotland. One possible exception is where crops are produced under protected conditions, such as glasshouses. *Meloidogyne enterolobii* has been detected as present and causing damage in two glasshouses in Switzerland (Kiewnick *et al.,* 2008; https://gd.eppo.int/taxon/MELGMY/distribution/CH), illustrating the potential for establishment via this route.

Many of the temperate species that are either present, or that could potentially be introduced, are either rare or have host ranges that do not include economically important crops or infect crops that are not grown in Scotland. The risk from these species can therefore be viewed as minimal. The main potential risks in terms of Scottish crop systems are most likely to be posed by species that are adapted to the climate and are either present in continental Europe or already detected in the UK. These species are *M. fallax, M. chitwoodi, M. minor, M. hapla, M. naasi and M. artiellia.*

3.7.1 *Meloidogyne fallax and M. chitwoodi*

Perhaps the greatest RKN threat to potato production in Scotland is represented by *M. fallax* and *M. chitwoodi*. Both species are present in continental Europe (Figure 10) and the threat posed by these species is recognised by EPPO, who have a national regulatory control system available (EPPO, 2013).

Three detections of *M. fallax* in England have been reported. In 2013 it was detected on leeks and in the margins of the infected field on various weeds and wildflowers. In 2018 and 2020 it was detected on organic carrots and in both cases was also present on volunteer potatoes in the field. In all three cases infected vegetable waste applied as an organic amendment was suggested as the likely route for introduction. *Meloidogyne fallax* has also been found on pitch turf in various football stadia. In all cases, DEFRA has imposed improved management practices to avoid spread of the nematode. Following the EU and due to it being present in England, *M. fallax* is now a regulated non-quarantine pest (RNQP) for Great Britain under GB Plant Health Legislation and statutory action would be taken should *M. fallax* be found in association with seed potatoes.

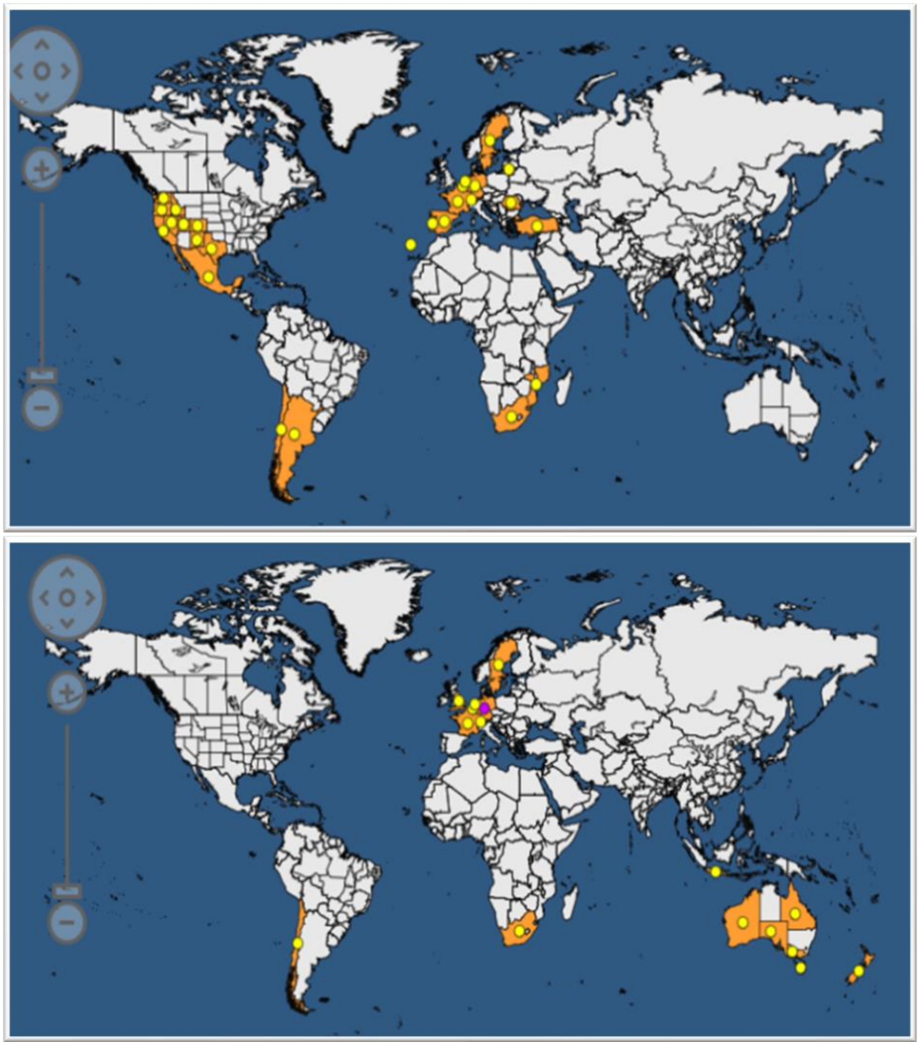

Figure 10 - Global distribution of M. chitwoodi (top panel) and M. fallax (bottom panel). From EPPO global database (www.eppo.int), November 2023.

Surveys have been undertaken in the UK to identify the species of *Meloidogyne* present. These have used samples collected during surveys for ring rot and brown rot. No infestations of *M.*

chitwoodi have been detected in these surveys. Surveys of carrot showing symptoms of root-knot nematode infection have identified *M. hapla*; no *M. chitwoodi* has been identified in these analyses. The current status of *M. chitwoodi* is therefore considered absent in the UK as confirmed by survey. *M. chitwoodi* is a Regulated Quarantine Pest. As for *M. fallax, M. chitwoodi* has been recorded extensively in continental Europe. In response to the threat posed by *M. chitwoodi* the UK Plant Health Authority have recently published a pest specific plant health response plan for this nematode on the UK Plant Health Portal (https://planthealthportal.defra.gov.uk/assets/Contingency-plans/M_chitwoodi_CP_v2024.pdf). The plan provides details of the response to be undertaken in the event of this nematode being detected in a potato crop in the UK.

The evidence to date suggests that the measures in place are sufficient to prevent entry of these pathogens. Given the huge damage potential of both these RKN species it is critical to ensure that these measures are maintained.

3.7.2 Meloidogyne minor

Meloidogyne minor is widespread throughout the UK, with grasslands and dune areas thought to be the natural habitat of this species (Prior *et al.,* 2015). It has been identified as causing damage to amenity turfgrasses, most notably golf courses and football pitches (Fleming *et al.,* 2016). It is possible that these infections have resulted from use of coastal sand for construction of the grass surface. Studies in Belgium indicate that *M. minor* has the potential to cause damage to potato, even at low population levels (Wesemael *et al.,* 2014). However, no reports of damage to potato in Scotland were found during the course of this review. It is possible that if pasture land is converted for use with potato, possibly as a result of other land previously used for potato production being infested with PCN, that *M. minor* may become an issue. There may also be a risk from infested soil associated with damaged amenity turfs should such material be transported to farming areas. However, such occurrences are extremely rare. Given that *M. minor* is native and is clearly causing little damage at present, the risk from this species can be considered as low.

3.7.3 Meloidogyne hapla

Meloidogyne hapla is apparently widespread throughout the UK and is described as causing damage on various ornamental plants, with occasional reports of damage to root crops such as parsnips and carrots. It has long been known that *M. hapla* can cause serious damage to potato (*e.g.* Griffen & Jorgensen 1969), illustrating the potential for this nematode to impact Scottish potato production. However, such damage is not observed, even with regular official inspections in place. There are several potential explanations as to why damage on potato might be limited. First, many grasses and cereals appear not to be hosts for *M. hapla*, and the inclusion of cereals in typical rotations in Scotland may therefore help suppress populations. Secondly, nematicides are frequently applied to other crops in a typical rotation, including parsnips and carrots, as protection against *Pratylenchus* spp. and virus vector nematodes. This may also help suppress *M. hapla*. Many effective nematicides are now being withdrawn and it is possible that one consequence of this may be to increase the prevalence of, and damage caused, by *M. hapla*.

3.7.4 Meloidogyne naasi

Although *M. naasi* is not a pathogen of potato, it has been noted in a cereal crop in Scotland (K. Davie, pers comm). Where cereals are grown repeatedly it has the potential to build up to damaging levels. It should also be noted that damage caused by *M. naasi* is likely to be exacerbated by heat stress, as seen for other nematode pathogens of cereals (*e.g.* Smiley *et al.,* 2017). Changes in patterns of nematicide use coupled with climate change may therefore increase prevalence of *M. naasi* and damage on cereals.

3.7.5 *Meloidogyne artiellia*

This RKN species is present in the UK but no reports of damage occurring in the UK were found during the present study. It is unlikely that this RKN species will emerge as a significant potato pathogen in Scotland.

3.8 *Control options*

It is important to note that many species of root-knot nematodes have broad host ranges that include several crops widely used in Scottish farming systems. It is therefore extremely difficult to manage populations of root-knot nematodes using rotations in the way that is possible for PCN.

3.8.1 *Statutory monitoring and regulation*

For the most potentially damaging species the best control option will be to ensure that the nematodes are not introduced into Scotland. As for PCN, the most likely route for introduction of *M. chitwoodi* and *M. fallax* is through infested seed potato. Detection of infested potato is based on visual inspection of the seed itself or inspection of host plants grown in the same field used for production of the seed potato. Symptoms are not always visible, so extreme caution is advised when importing seed tubers from a region in which these nematodes are known to be present. Additional options for detecting RKN on potato are available, including analysis of nematodes extracted from tuber peel (Viane *et al.,* 2007), but these are extremely labour intensive. The ban on import of Solanaceous species into Europe is helpful in minimising introduction of other RKN species currently not present in Europe.

3.8.2 *Nematicides*

Nematicides have traditionally formed the bedrock of control strategies for RKN, but few active ingredients are now available to growers. Fluopyram (sold as Velum Prime) is licenced for use on carrots and potato and, as stated above, may help to suppress native RKN species when applied for control of free-living nematodes.

3.8.3 *Hygiene*

As described above, the outbreaks of *M. fallax* in England may have occurred after application of composted vegetable waste, while problems due to *M. minor* are reported to be associated with inappropriate disposal of heavily infected amenity grasses. Avoiding the use of non-assured compost sources is therefore recommended. Movement of farm machinery across these areas may represent a potential risk of spreading this nematode. *M. minor* spread potentially via movement of sand from its natural habitats is another useful illustration of the risks inherent in moving sands, soils and aggregates.

3.8.4 *Cover crops*

A greater awareness of the potential benefits of regenerative agriculture has led to an increase in the uptake of cover crops in these systems. Radish is widely used in these systems for the perceived benefits of its deep tap roots. Cover crops, including varieties of fodder radish that are partially resistant to *M. fallax* or *M. chitwoodi* are widely used in continental Europe as part of integrated management strategies and extensive analysis of the ability of such crops to provide control of root-knot nematodes has been undertaken. For example, a recent study showed that several varieties of fodder radish (‘Maximus’, ‘Contra’, ‘Dacapo’ and ‘Defender’) were poor hosts for *M. chitwoodi* and suppressed populations in field studies (*e.g.* Taning *et al.,* 2023). However, little is known about how such crops perform in Scottish conditions. In addition, some cover crops may allow substantial reproduction of other nematodes, including free living nematodes (*e.g.* Grabau *et al.,* 2017; Taning *et al.,* 2024). Care is therefore needed in use of cover crops.

3.8.5 Natural resistance

Breeding programmes for resistance against both *M. fallax* and *M. chitwoodi* are in progress in continental Europe and the Pacific Northwest of the USA. Resistance has been identified in several wild relatives of potato including *Solanum bulbocastanum, S. cardiophyllum, S. brachistotrichum, S fendleri* and *S. hougasi (*Janssen *et al.*, 1995) and partial resistance to *M. chitwoodi* is available in a limited range of cultivars (*e.g.* Norshie *et al.,* 2011). However, no cultivars with complete resistance to *M. chitwoodi, M. fallax, M. hapla* or *M. minor* are currently available to growers.

3.9 Knowledge gaps

- A detailed analysis of the distribution of non-regulated native and established species of root-knot nematodes of the highest economic importance in the UK is still lacking. Given changes in the use of nematicides that are currently being imposed due to legislative pressures, as well as changes in the UK climate, the absence of such information makes assessment of the likely risks posed by these species extremely difficult to calculate.
- It is critical to remain vigilant and to ensure training remains up to date among those undertaking inspections. Ensuring awareness of symptoms of root-knot nematode infection, particularly among those undertaking inspections, would potentially help in early awareness of the presence of root-knot nematodes.
- The effectiveness of cover crops against RKN under UK conditions is not well understood. If available nematicides continue to be withdrawn it will be essential to provide growers with information about alternative control strategies, including cover crops. Some widely grown species of cover crops may well be hosts for some RKN species, or for free living nematodes. Similarly, little is known about the impact of wildflower mixes on RKN populations. Further study of this area is therefore merited.
- Although some potato varieties with partial resistance are available, there are currently not sufficient options available for growers should and RKN species become a major problem in Scotland.
- It would be advantageous to consider the feasibility of developing screening and diagnostic processes to identify RKN pre-planting (as with PCN), as a supplement to official inspection controls for regulated RKN species.

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