Project title:	Carrots & Parsnips – developing a strategy to control free-living nematodes
Project number:	FV 447
Project leader:	Dr. Roy Neilson, The James Hutton Institute, Dundee, DD2 5DA
Report:	Final report, 2016
Previous report:	None
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Date project commenced:	1 April 2016
Date project completed:	3 September 2016

DISCLAIMER

AUTHENTICATION

I declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

A significant knowledge gap exists with regard to the impact of free-living nematodes (FLN) on carrot and parsnip production. A lack of available land to increase rotation length and considerable use of rented land are significant constraints for deploying novel strategies to manage free-living nematodes.

Background

For decades, commercial practice to manage free-living nematodes has been the application of synthetic chemicals. However, in recent years this practice has been challenged through legislation focussed on reducing the majority of active ingredients, including those currently available to manage nematodes. Furthermore, the societal push for sustainable agricultural production minimising environmental impacts, yet maintaining current yield through a balance of inputs, is an additional major driver. Thus, alternative management strategies for free-living nematodes are required. In the context of the carrot and parsnip sector, free-living nematodes have not been deemed a priority area for research funding. Available underpinning data, in an UK context, has primarily been provided through pot experiments which have limited relevance to field conditions. Thus the relatively narrow timeline for active ingredient removal has left the sector potentially vulnerable until alternative effective strategies for nematode management are identified and established. This situation was brought into sharp focus with recent production problems associated with one of the leading products in the marketplace. This led to an unexpected shortage of supply across numerous crop sectors and provided focus on how to address the implications of legislation to reduce available active ingredients.

Consequently, this review was initiated with the remit to collate previous work on free-living nematodes associated with carrot and parsnip production and to look beyond the UK and identify management practices that are being investigated and/or deployed elsewhere (potentially in other crops), and to highlight their potential or otherwise for the carrot and parsnip sector. Based on the review, seven action points have been identified for consideration by growers and agronomists with five topic areas recommended for research funding that have potential for contributing to long-term sustainable production of carrot and parsnip.

Summary

It is evident from the literature that significant knowledge gaps are present with regard to the impact of free-living nematodes associated with the production of carrot and parsnips in the UK. Beyond the UK, considerable research effort has been undertaken to determine alternative strategies for managing free-living nematodes. This study has evaluated the various strategies noting both advantages and disadvantages. A summary of the different strategies with likely useable timelines is presented in Table 1.

Table 1. Summary of available potential alternative management strategies for management of free-living nematodes based on literature analysis, noting availability of UK based data, potential for success and potential deployment timeline.

Management strategy	UK data available	Potential for success	Deployable timeline
Breeding for resistance	No	Limited given grower concerns	Long-term
Rotation	Yes	High	Immediate
Monitoring for free-living nematodes	Yes	High	Immediate
Biofumigation	Becoming available	Unknown – results to date inconsistent	Medium
Green manures and cover crops	No	Medium	Medium to long-term
Tillage	Limited	Medium	Short-term
Soil amendments	Limited	Good	Immediate to medium-term dependent upon the specific amendment
Biological control	Limited	Low to medium	Long-term with possible regulatory hurdles
Arbuscular mycorrhizal fungi	Yes	High	Medium

Ultimately, uptake and adoption of any or all of the above management strategies will be balanced against economic gain in terms of yield and quality improvement, traded off against cost of deployment. Furthermore, a barrier to adoption of new management strategies may simply be that other pest and pathogens are deemed to be more economically important than free-living nematodes. These decisions are likely to be made at the resolution of the individual farming unit rather than sector wide.

Financial Benefits

One of the few tangible costs that can be calculated is if intensive soil sampling is used for assessment of free-living nematodes using state-of-the-art molecular diagnostics, the cost per hectare to the grower would be in the region of £70-£100 depending upon the selected service supplier.

It can be envisaged that going forward rented land with a known rotation comprising of few crops deemed as a good host for free-living nematodes could attract a premium rent. In addition, if fields had been subjected to pre-plant testing for FLN and shown to be FLN free or in low numbers a substantial premium could be levied on rental fields. Such an additional potential cost is intangible and would need to be balanced against economic loss in terms of yield and quality.

As limited underpinning knowledge of the economic impact of free-living nematodes on carrots and parsnip production in the UK has been published in the peer-reviewed or grey literature it is difficult to determine the financial benefit to be accrued from the stated recommendations. Whilst many practitioners have excellent farm records of quality and yield, at best symptoms can only be anecdotally attributed to free-living nematodes.

However, based on 2015 figures (British Carrot Growers' Association), 700,000 tonnes of carrots were produced from 9,000 ha with a marketplace value of *c*. £290M. Thus, based on typical damage levels of FLN across a range of crops, an estimate of an average 10% reduction in yield and/or quality due to the impact of free-living nematodes would result in an approximate minimum £29M annual cost to the sector. Using conservative values determined for other crops, if a number of alternative management strategies were used in line with the long-term recommendations, it can be speculated that free-living nematode could be reduced in the region of 10-20%, i.e. yielding a saving of in the region of £3-6M annually. It cannot be emphasized enough that these figures are speculative and should be considered with extreme caution.

Action Points

Potential actions to achieve short-term benefits include:

i) Where possible increase length of rotation, even an additional one year may be beneficial.

ii) Ensure that rotation crops include those that are poor hosts of FLN that impact carrot and parsnips, thus limit where possible, sugar beet, peas, beans and potatoes in the rotation which are known to increase damaging FLN.

iii) Monitor FLN by soil testing using where possible molecular diagnostics to minimise costs and improve specificity of testing.

iv) Optimal sampling for FLN monitoring represents a single composite sample per hectare. Each composite sample should comprise a minimum of 70 cores, taken to a depth of approximately 20 cm, collected randomly along a W shape walk.

v) Apply organic matter prior to planting.

vi) Where land is owned, rather than rented, consider inclusion of a cover crop within the standard rotation.

vii) Be alert to the presence of root-knot nematode and take appropriate measures to minimise spread.

SCIENCE SECTION

Introduction

Historically within the UK, the nematological focus in an agricultural context has for decades been centred on potato cyst nematode (PCN) to the near exclusion of other economically damaging nematodes. Thus, so-called free-living nematodes have with few exceptions e.g. soft fruit and diseases associated with viruses vectored by nematodes have on the whole been ignored. Whereas PCN represents two nematode species, *Globodera pallida* and *G. rostochiensis*, the term free-living nematode (FLN) encompasses numerous nematode species that cause yield or quality losses in crops. Such a loose terminology can cause confusion to non-specialists as FLN can represent different nematodes when dealing with different crops and thus unlike PCN, the acronym does not consistently represent the same nematodes thus crop context is crucially important.

However, consistent for FLN is their life cycle. Unlike PCN, no component of the life cycle of FLN occurs within the plant host. Thus reproduction takes place external to the plant host in soil. Therefore all cyst nematode belonging to *Globodera* (e.g. PCN) and *Heterodera* (e.g. carrot cyst nematode, *H. carotae*) are not and should not be classified as FLN. Similarly, species of *Meloidogyne*, commonly referred to as root-knot nematode (RKN), have a significant component of their life cycle within the host plant and unequivocally are not FLN. Hence, cyst nematodes and RKN are not within the scope of this study. However, it would be remiss not to note that beyond the UK (e.g. mainland Europe), RKN is the most damaging group of nematodes associated with carrots and significant economic investment and concomitant research has been directed on this group of nematodes associated with root vegetables and carrots in particular. Whilst firmly outside the project scope, RKN will be included later in this report under the future research section.

For absolute clarity and to provide context for this report, here FLN refers to species of nematode belonging to the following nematode genera: *Longidorus*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus* and *Trichodorus*. There is a further propensity in the literature and by agronomists to refer to FLN in descriptive terms such as “pin”, “stubby root” or “needle” nematodes which are most unhelpful as these terms can in different countries/regions refer to different FLN groups. Thus, it cannot be emphasised enough that care should be employed when using the term FLN and understanding the crop context.

Whilst the reproductive cycle of FLN is consistent, their biology can differ. In the context of carrot (and other crops), species of *Longidorus*, *Paratrichodorus*, *Paratylenchus*, and *Trichodorus* feed directly on the root system and root hairs thus are known as ectoparasites. Such feeding gives rise to characteristic symptoms (Fig. 1) where FLN can cause direct damage by a) reducing yield as a consequence of feeding on roots and altering water and nutrient relations or b) reducing quality due to malformation of the tap root (fanging) e.g. carrot and parsnip. *Pratylenchus*, however, are semi-endoparasites and thus have the ability to briefly enter the root cells and on egress, the entry point forms a wound that is frequently utilised by secondary pests and pathogens to facilitate the primary disease. This system is well characterised for potato (for example, MacGuidwin and Rouse, 1990; Forge et al., 2015) but not for carrot.

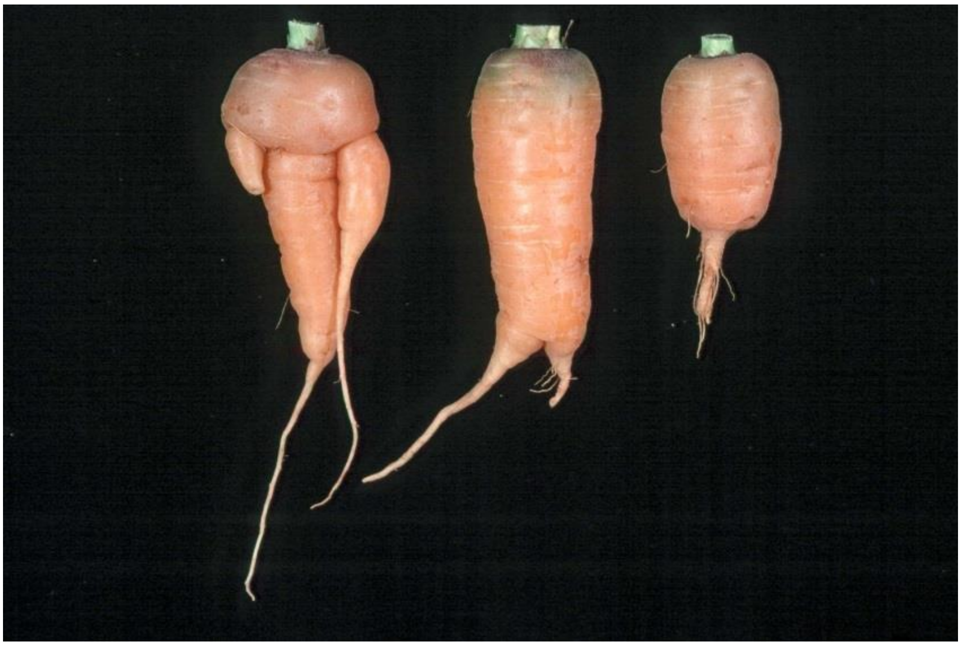

Figure 1. Characteristic mis-shape due to putative of free-living nematodes on roots

Soil-borne pests such as nematodes are major constraints to profitable crop production in the UK. In particular, FLN are emerging as a major problem for example in the UK potato (Dale & Neilson, 2006) and horticultural industries, exacerbated in the short term by removal of approved nematicides (91/414/EEC) and in the long-term by expected population increases due to climate change (Neilson & Boag, 1996). FLN have been calculated to consume approximately 10% of global agricultural output, causing economic losses valued at over $125 Billion annually (Chitwood, 2003). In the UK, arguably the adoption of set-aside in the 1990s provided a stable habitat with a diverse host range for FLN to thrive over a 1,3 or 5 year period (Boag et al., 1998) that resulted in increased FLN numbers. Once land in set-aside was returned to production, the increased FLN abundance coupled with reduced and inappropriate rotations (including susceptible host crops) further exacerbated FLN numbers. The lack of comparative focus on FLN and serendipitous secondary management through treatment of other pests and pathogens (e.g. nematicidal application for PCN) rarely brought FCN into sharp focus unless symptoms were extreme.

However, EU policy focussed upon significant reductions in pesticide use has potential to expose a range of UK crops, including carrots, to uncertain pressure as no replacement management strategies have been knowingly explored. Current European Directives on pesticide registration and usage has already resulted in the loss from the UK market of some key active ingredients to manage nematodes. The 'Thematic Strategy on the Sustainable Use of Pesticides' (EC, 2004) outlined a number of actions for the consideration of member states in order to achieve a declining trend in pesticide use. Under the Sustainable Use Directive (2009/128), these options will be further reviewed by the EU (Hillocks, 2012). Globally, a range of IPM strategies were reviewed and demonstrated that IPM can both reduce pesticide use and increase yields of most major crops. On average, yields increased by 40% and pesticides were reduced by 60% (Birch et al., 2011) thus demonstrating that opportunities exist for effective disease management under a challenging landscape but carrots were not included in this study.

Currently the primary product for putative FLN management in a range of crops is Vydate (a/i oxamyl). This product has been extended for use in the UK marketplace until 2018; however recent technical issues in production with minimal product availability in the UK have demonstrated the exposure of the UK agriculture and horticulture industry to FLN. It is unclear whether the product will be registered after this date. This has provided an early warning to the UK agricultural and horticultural industries of potential future production problems in the context of current products being removed from the marketplace. Recently, garlic based products have emerged in the marketplace for control of FLN. No peer-reviewed publications were found detailing the impact of such products of FLN in crops.

In stark contrast to Europe and North America, UK agriculture has been slow to conduct research and uptake alternative forms of in-field FLN management. To date, the limited research in the UK has been focussed on the potential pest management effects that field grown mustards offer which is commonly used in North America to control FLN. Having a potential single solution replicates the current situation of reliance on synthetic chemicals and is unsustainable in the long-term. Thus a range of achievable strategies are required to maintain long-term sustainable agricultural production.

Therefore there is an urgent imperative to understand current knowledge on FLN associated with carrot (and parsnip) production as follows:

1. A holistic synthesis review of the scientific and grey literature, building upon a previous review (FV 232), to ascertain a rounded understanding of the current knowledge of FLN associated with carrots and parsnips targeted to a range of stakeholders;
2. Present a robust and effective management strategy or strategies to manage FLN to facilitate sustainable UK carrot and parsnip production including recommendations for future research and knowledge transfer.

Materials and Methods

This study utilised two separate strands for collation of information. Firstly, to understand existing knowledge of FLN associated with carrot and parsnip production, a collection of > 4000 peer-reviewed articles on FLN that the author curates were consulted. In addition, to ensure a wider coverage of FLN research beyond the UK, scientific databases such as Web of Science, Scopus and Google Scholar were interrogated as were policy databases e.g. European Union.

Secondly, face-to-face discussions were held with individual agronomists, clients of the James Hutton Limited nematode diagnostic unit, individual growers through personal networks and AHDB facilitated meetings. Further and valuable face to face discussions were held with a subset of delegates to the British Carrot Growers Association AGM. Electronic contact through email was held with numerous nematology colleagues across Europe and North America who have expertise in managing FLN associated with vegetable production.

Results and Discussion

Known FLN associated with Carrots and Parsnips

The paucity of published peer-reviewed experimental field data on UK field vegetables is illustrative of the previous lack of focus on FLN. National field surveys (Boag, 1979, 1980) reported that UK fields, comprising light sandy soil consistent with the known association with trichodorids (Taylor and Brown, 1998), used in rotations that included carrots and parsnips had numerous potentially damaging FLN species belonging to the following genera: *Trichodorus*, *Paratrichodorus*, *Longidorus*, *Pratylenchus*, *Bitylenchus*, and *Merlinius*. However, at these sites no determination of yield or quality effects was done. Approximately thirty years later (2002), a review and investigation of factors influencing crop damage by plant-parasitic nematodes (FV 232) in carrot and parsnip was in agreement with that of Boag (1979, 1980) regarding the key FLN species/genera associated with carrot and parsnips. The same review (FV 232) investigated temporal sampling to identify the optimal sampling point for FLN. However, possibly due to the significant constraint of low sample number and lack of disparate geographical sampling, results were inconclusive. The same review (FV 232) explored threshold values of *Trichodorus* and *Paratrichodorus* species in relation to carrot fanging and yield using a pot-based experimental design. Similarly, pot tests were deployed to explore the interaction between FLN and leeks (2013, FV 377a). It is well recognised that *Trichodorus* and *Paratrichodorus* are exceptionally sensitive to artificial environments and it is unclear from the review whether the authors undertook mitigating strategies to minimise the stress and thus ensure typical FLN activity were as found under normal field conditions (see FV 249). More recently, in Germany Teklu et al. (2016) explored yield and quality losses associated with *Pratylenchus penetrans* again using a pot based experiment. These authors ascertained that 100 *P. penetrans* 200 g^{-1} soil was the threshold limit for negative impacts on carrot yield. However, the authors noted that field trials should be held to clarify whether their pot results adequately translated to field conditions. Pot tests are not indicative of field conditions as soil has to be packed into pots, rarely if ever matching the soil matric potential of field soil (Yeates et al., 2002) a key driver of nematode motility in soils. Hence, pot tests can be at best described as illustrative rather than informative.

Notwithstanding the constraints of pot tests and limited field studies within the UK and beyond, a consensus list of FLN species frequently reported as associated with carrots that are known to be present in the UK can be established (Table 2).

Breeding for resistance

The cornerstone of any strategy for sustainable crop production is the initiation and adoption of breeding programmes for resistance. A recent example of successful breeding to overcome a nematode problem is the development of resistant varieties of sugar beet to sugar beet cyst nematode (**www.bbro.co.uk**). Whilst not FLN, resistance to the RKN species, *Meloidogyne hapla* has successfully been incorporated into carrot in North America (Frese, 1983; Wang and Goldman, 1996). However this requires significant economic investment by the industry with likely benefits not achievable within a decade. Furthermore, vociferous concerns were raised by a number of key UK growers during face to face discussions as a belief exists that consumers would not move away from the current 'Nantes' type carrot which has dominated the UK market since the 1970s. Therefore an *en passe* is evident regarding what is recognised as best practice for a long-term strategy of sustainable crop production and likely adoption by UK growers. One possibility is an education programme for consumers but clearly this would be a resource heavy task for a relatively low margin industry. Thus, alternative strategies need to be identified and explored.

Table 2. Primary FLN species derived from the literature that impact upon carrot production and availability of molecular or standard tests

FLN species known to impact carrot	Present in UK	Molecular	Standard
Longidorus elongates	Yes	Yes	Yes
Paratrichodorus anemones	Yes	**Yes***	Yes
Paratrichodorus pachydermus	Yes	**Yes***	Yes
Paratylenchus nanus	Yes	No	Yes
Paratylenchus bukowinensis	Yes	No	Yes
Pratylenchus penetrans	Yes	**Yes**	Yes
Pratylenchus neglectus	Yes	**Yes**	Yes
Trichodorus primitivus	Yes	**Yes***	Yes
Trichodorus similis	Yes	**Yes***	Yes
Tylenchorynchus dubius	Yes	No	Yes
Tylenchorynchus maximus	Yes	No	Yes

Species in bold are known to have a widespread UK distribution. Molecular = molecular diagnostic indicating presence/absence, those in bold also provide quantification. Standard = standard microscopy based identification. * = exclusive to James Hutton Institute. Other FLN species that have been reported to affect carrot but have a restricted geographic distribution within the UK are not listed here.

Rotation

A relatively straightforward management option for growers is to both extend the rotational period between each carrot and parsnip crop and ensure that the crops used within the rotation are non-hosts of FLN that can cause damage. Optimum rotation would be around seven years with a number of cereal crops included in the rotation. During face to face discussions with growers it became apparent that rotational periods varied between 3 and 8 years and that suitability of crops used within the rotation differed based on geographic location. Thus in Scotland, barley was a common crop in the rotation and is not a good host for many of the FLN that impact carrot and parsnip. In contrast, in East Anglia rotational crops frequently included sugar beet, potato and beans which are known to be excellent crops for maintaining and increasing populations of FLN. As Hoek et al. (2013) notes a poor rotational choice of crops can result in > 30% reduction in carrot yield.

It was also noted that for many growers, it was impossible to improve the rotational period for carrot and parsnip growing due to the lack of available suitable land for the two crops. Thus, with time, the constraint of land availability will also have an impact and likely increase FLN abundance unless mitigating strategies are deployed. No published data on FLN could be found but Belair and Parent (1996) demonstrated that a longer and diverse rotation significantly increased carrot yield when challenged with RKN.

Monitoring for FLN

Monitoring is one of the key principles of effective integrated pest management and cannot be implemented effectively without accurate estimates of target nematode abundance; assessment of presence/absence of natural control; or without reliable assessments of crop damage and its effects on yield. Furthermore, long-term baseline data should be available to act as a direct comparator of field collected data.

The amount and frequency of monitoring required for decision making depends upon the crop and its pathogens. Almost invariably, uniformity of pathogen infestations does not occur, so it is essential to take a representative sample that overcomes the lack of uniformity. Also, it is important to make a representative survey of a field in the least amount of time.

The resolution of monitoring is dependent upon background knowledge of the a) target crop; b) biology of the pathogen; c) main soil factors suitable for pathogen development and d) environmental factors e.g. weather conditions.

Key to monitoring is appropriate sampling given that soil borne pathogens have a heterogeneous, i.e. patchy distributed within fields even at small scale especially true for FLN (Boag et al., 1996; Nielsen et al., 2010). The vertical profile of FLN should also be considered when sampling as different species are known to inhabit soils at differing depths. For example, *Longidorus elongatus* is generally more abundant at the soil surface, *Trichodorus* and *Paratrichodorus* are known to be recovered over a range of depths reflecting the root system of the plant or soil conditions such as moisture (Alphey, 1985; Boag et al., 1989). *Pratylenchus* have been reported to have high abundance in root zone areas within soil (Pudasaini et al., 2006). Trichodorids have been known to follow the water table with a vertical movement of up to 90 cm in sandy soil (Boag, pers comm.). Furthermore, nematodes are known to move via lateral flow across soil as a consequence of rainfall events (Baxter et al., 2013). This known movement of trichodorids with the water table does raise questions regarding the impact of irrigation application and timing and whether there is an unintended consequence of contributing to crop disease in carrots by in effect reducing the distance between the crop and FLN burden at the most vulnerable stage of plant growth. Whilst localised spread of FLN has been reported to be aided by farm machinery (Boag, 1985) and other nematodes dispersed by wind in light sandy soil (Carroll and Viglierchio, 1981; Andrade and Asmus, 1997) limited research has been published in these areas. Perhaps, this is a direct consequence of Taylor et al. (1994) who demonstrated at two sites that after 30 years of commercial practice in-field FLN distribution was on the whole similar.

Thus the choice of sampling technique for field testing is a vital consideration. Using a diagonal transect across a field is applicable for general surveys. For FLN, a W pattern with samples taken at random points (Marshall et al., 1998) with a minimum of 70 sub-samples (cores) is required and ideally one composite sample per hectare. Notwithstanding the optimum sampling strategy, ensuring the correct sample size is important to fully exhaust the potential for nematode detection. For example, the traditional standard soil sampling unit for extraction of nematodes is 200 g (Flegg and Hooper, 1970) which has recently been confirmed in a molecular context (Wiesel et al., 2015). Such a large sample size conflicts with the volumes of soil that DNA extraction kits typically use, e.g. 0.5-10.0 g. Furthermore, DNA extraction directly from soil can be confounded by the presence of inhibitory factors though methods are being developed to overcome this barrier (Brierley et al., 2009; Woodhall et al., 2012).

With the increasing availability of high-throughput DNA based diagnostics, pre-plant testing for FLN becomes a realistic option for the industry. For example, DNA diagnostics for four trichodorid species (Table 2) have been developed and commercialized through InnovateUK funding (project number TP 292-249). Similar diagnostics are expected to be developed in the coming years through an AHDB studentship (project number PHD132) for *Pratylenchus* species that are relevant to carrot. Thus in the short term the UK carrot industry will have access to state-of-the-art FLN diagnostics that will permit management decisions to be made on whether a field could be used for carrot and parsnip production. An initial economic outlay (likely in the region of £50-100 per sample, dependent upon sample number) to generate a field map (e.g. Figure 2) is therefore feasible. In addition, such an analysis is likely to be relevant for at least two full crop rotations as FLN are known to have limited movement over long periods of time (Taylor et al., 1994). Integrating FLN monitoring into a precision agriculture framework provides a potential opportunity to deploy additional management strategies at those areas in the field with elevated FLN abundances. Thus, generation of such field maps allows the development of multi-layered IPM strategies that could conceivably include for example, a field scale application of organic matter combined with localised cover crops or non-host crop that encompass the areas of high FLN abundance to have a suppressive effect and minimise FLN impacts on future carrot and parsnips. Consideration would need to be made with regard to economic trade-offs to ensure that such action was cost effective to the individual farm unit.

An effective way forward would be to integrate nematode data into decision support systems (DSS) and where possible precision agriculture methodologies. Examples of the former are NemaDecide (Been et al., 2007) and BCN-Watch decision support systems for the management of potato and beet cyst nematodes in the Netherlands and Sweden respectively.

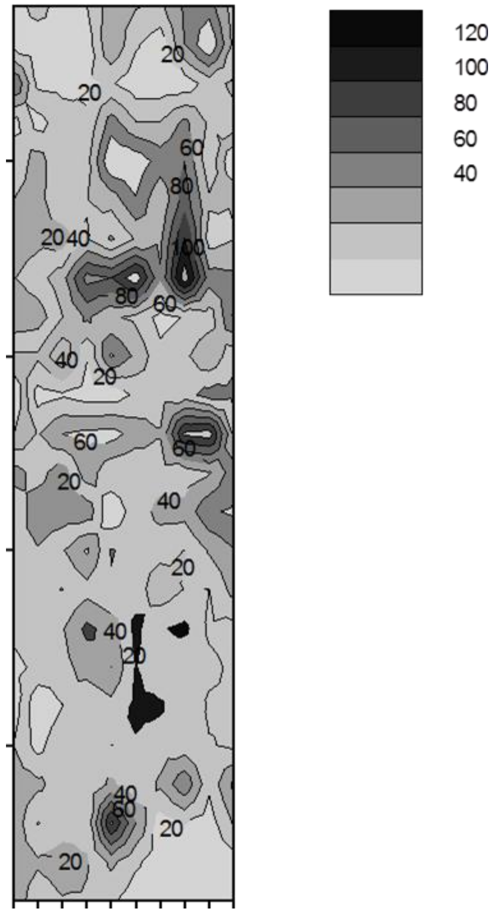

Figure 2. Exemplar high resolution distribution map of trichodorid nematodes derived from intensive soil sampling. Darker colours represent higher nematode abundance and are expressed as abundance per 200 g of soil.

However, the industry has a long held concern regarding the utility of FLN diagnostics as results have been inconsistent. This has not been helped by the various service providers of FLN diagnostics in the UK using a range of different nematode extraction methods, many of which are frequently inappropriate for the various target nematode species (Brown and Boag, 1988; Neher et al., 1995). Variables that can impact upon diagnostics include depth of sampling, time (of year) of sampling, appropriate sampling method, size of sample taken, and heterogeneity of FLN field distribution and extraction method. It was established in the 1960s and 1970s that optimal sampling for FLN is at a depth of 0-15 cm and that time of sampling should be spring or autumn. Yet this key information has not been translated to the industry as this was only dealt with in 2012 (FV 377).

Biofumigation

In recent years there has been much interest in the potential of so-called biofumigant crops belonging to the family Brassicaceae. Such cruciferous species produce significant levels of glucosinolates (GSLs) held in plant cells separate from the non-toxic enzyme myrosinase (Manici et al., 1997). However, when plant cells are ruptured the compounds come into contact and are hydrolysed in the presence of water to release isothiocyanate compounds (Vig et al., 2009). These compounds have a wide range of biocidal characteristics and are acutely toxic to a variety of pests and pathogens (Chew, 1987) including nematodes (Avato et al., 2013). Common biofumigant crops include: *Brassica juncea* (Brown mustard), *Brassica nigra* (Black mustard), *Eruca sativa* (Rocket), *Raphanus sativus* (Radish) and *Sinapsis alba* (White mustard). To date in the UK, biofumigant crops have generated inconsistent results against FLN across a number of crops, however they were successful in helping to manage tobacco rattle virus vectored by *Paratrichodorus* and *Trichodorus* species in potatoes (InnovateUK project number TP 292-249). A BBSRC-HAPI funded project (project number N1700602) will explore at field level the efficacy of biofumigant crops against FLN in carrots. Soil samples have been collected during the 2016 season and the first results known soon thereafter. Biofumigant crops can be used in a number of different ways for FLN management:

Intercropping and rotations with biofumigants

Above-ground plant material is harvested and hence activity against FLN relies on GSLs or other compounds released through leaf washings or root exudates. Several studies have detected both GSLs and isothiocyanate compounds in the rhizosphere which have been implicated in the suppression of pests and pathogens (van Dam et al., 2009). Moreover, GSLs and isothiocyanate compounds can affect the composition of rhizosphere communities which may also suppress soilborne plant diseases and some common beneficial microbial species such as *Trichoderma* show high tolerances to isothiocyanate compounds (Smith and Kirkegaard, 2002; Galetti et al., 2008; Gimsing and Kirkegaard, 2009).

Incorporation of biofumigants

This is the most recognised use of biofumigant plants where a crop is grown specifically for incorporation with the aim of converting GSLs to isothiocyanate compounds. This is achieved by comprehensive maceration of plant tissue followed by rapid incorporation into soil and addition of water if required to ensure complete hydrolysis (Matthiessen and Kirkegaard, 2006; Kirkegaard, 2009). As some isothiocyanate compounds are volatile, sealing/smearing the soil with a roller or covering the soil with plastic mulch may be beneficial (Kirkegaard and Matthiessen, 2004).

Seed meals and other processed biofumigants

Defatted seed meal produced after the processing of brassica seeds for oil (e.g. in mustard crops) also offer a convenient source of high GSL material for soil amendment (Brown and Mazzola, 1997). These materials have shown promise against a number of soilborne plant pathogens including *Rhizoctonia* spp. (Mazzola et al., 2007) and *Meloidogne* spp. (Lazzeri et al., 2009) but no reports for action against FLN were found. A liquid formulation has also been developed from defatted *B. carinata* seed meal which had activity against *Meloidogyne incognita* (De Nicola et al., 2012). Other products based on pellets of dried-high GSL plants have also been developed and showed good activity in vitro against *Pythium* and *Rhizoctonia* (Lazzeri et al., 2004). The main advantages of this approach are that these products can be used at times of year when growth of biofumigant plants is restricted (e.g. in the winter), can be more easily integrated in rotations, and are more amenable to intensive production systems where break crops are not used.

Green manures and cover crops

Cover crops and green manures are grown with no intention of harvesting their biomass, either partly or completely, at the end of the cropping season. The difference between these two types of crops is their final use. The above-ground part of green manures is incorporated into the soil at the end of the growing period with the aim of returning accumulated nutrients (e.g., nitrogen) or useful secondary metabolites (e.g., glucosinolates) to the soil. Cover crops are grown for different reasons, such as reduce leaching of nutrients (e.g., nitrate), avoid erosion, improve soil structure or suppress weeds. A combined use is also possible, a crop can serve first as cover crop (e.g., for weed control) and then be incorporated as green manure (e.g., for nutrient input) (Campiglia et al., 2009). One use of green manures/cover crops to control soil-borne diseases is their role as a non-host crop as part of a crop rotation strategy. Also specific brassica green manures have been used as trap crops for the control of nematodes (Jaffee et al., 1998) but have not been tested against FLN.

Cover crop roots can liberate a range of molecules (e.g., sugars, amino acids) and thus directly influence the community composition and biomass of soil microorganisms (Ladygina and Hedlund, 2010). Cover crops have been shown to influence the soil microbial biomass and community structure and have influenced the reduction of several soil-borne potato diseases by these crops (Larkin et al., 2010). A second way to reduce soil-borne pathogens through cover crops is the use of nematode catch crops, such as *Tagetes* spp. (Held et al., 2000) though with limited testing against FLN (Dover et al., 2003; Hook et al., 2010). So-called plant species diversity i.e. “the conservation or introduction of plant diversity in agroecosystems” has been advocated for the management of a range of pest and pathogens including nematodes (Ratnadass et al., 2012). As with other management strategies the focus of this research area has been PCN and RKN. It is clear from discussions with growers and agronomists that where land is rented addition of a cover crop would be difficult to achieve.

Tillage

Conservation agriculture has three key central tenets namely no- or minimal tillage, crop rotation and soil cover (Giller et al., 2015). Whilst the focus of such methods have previously been in tropical farming or large scale activities in Australia and North America, increasingly there is interest in its utility to contribute to pest and pathogen management including nematodes. For example, Brainard and Noyes (2012) reported that strip tillage (in combination with organic amendment) positively influenced carrot yield and quality with a degree of nematode management. There is still much research to be done in this area (Roger-Estrade et al., 2010) but the impact of tillage on FLN has been reported to be dependent on soil depth (van Capelle et al., 2012) and type e.g. minimum, zero tillage (Griffiths et al., 2012). However, in a recent EU study, carrot relevant FLN genera *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* (Table 2), did not differ in abundance as a consequence of tillage (minimum *vs*. plough) treatments but did alter due to crop host (Hallmann et al., 2015).

Soil amendments

Application of organic soil amendments is a long-standing, well understood and traditional method of obtaining nutrients for crop growth (Lemaire et al., 2014). However, with changing agricultural practices coupled with a challenging marketplace the integrated arable-livestock model has changed (Lemaire et al., 2014). In addition to enhanced nutrient status, organic amendments are recognized in having a role in the management of plant–parasitic nematodes (Oka, 2010). A variety of organic amendments, such as animal and green manures, compost, nematicidal plants and proteinous wastes, are used for this purpose, but efficacy of nematode management is inconsistent (Oka, 2010) and mulch has been shown to support four times as many *Pratylenchus* on carrot roots compared with a no mulch control (Holmstrom et al., 2008).

Biochar

Biochar is the solid material obtained from thermochemical conversion of biomass, typically plant material in an oxygen-limited environment. The utility of biochar as a soil amendment, a means to sequester carbon or a management strategy for soil-borne pest and pathogens has been much debated (Lehmann and Joseph, 2015). In a recent study of the impact of biochar application on the FLN species *Pratylenchus penetrans*, tap root infection rates of carrot by *P. penetrans* were significantly reduced by approximately 80% (George et al., 2016).

Compost

The suppressive capacity of compost against soil-borne pathogens, including nematodes, has been demonstrated in several studies, and, consequently, the use of disease suppressive compost can reduce crop losses and benefit growers (Noble and Coventry, 2005; Hadar, 2011). Compared to other amendments such as crop residues and peat, compost has been demonstrated to be more efficacious (Bonanomi et al., 2007). Suppressive effect of compost is generally proportional to the inclusion rate in soil and in combination with other management interventions such as tillage regime and cover cropping can enhance carrot yield (Brainard and Noyes, 2012). Success or failure of compost for disease control depends on the nature of the raw materials, on the composting process used and on the maturity and quality of the compost (Termorshuizen et al., 2006). Oka (2010) suggested possible mechanisms involved in nematode suppression through organic matter application including: a) release of pre-existing nematicidal compounds in soil amendments; b) generation of nematicidal compounds, such as ammonia and fatty acids, during degradation; c) enhancement and/or introduction of antagonistic microorganisms; d) increase in plant tolerance and resistance; and e) changes in soil physiology that are unsuitable for nematode behaviour.

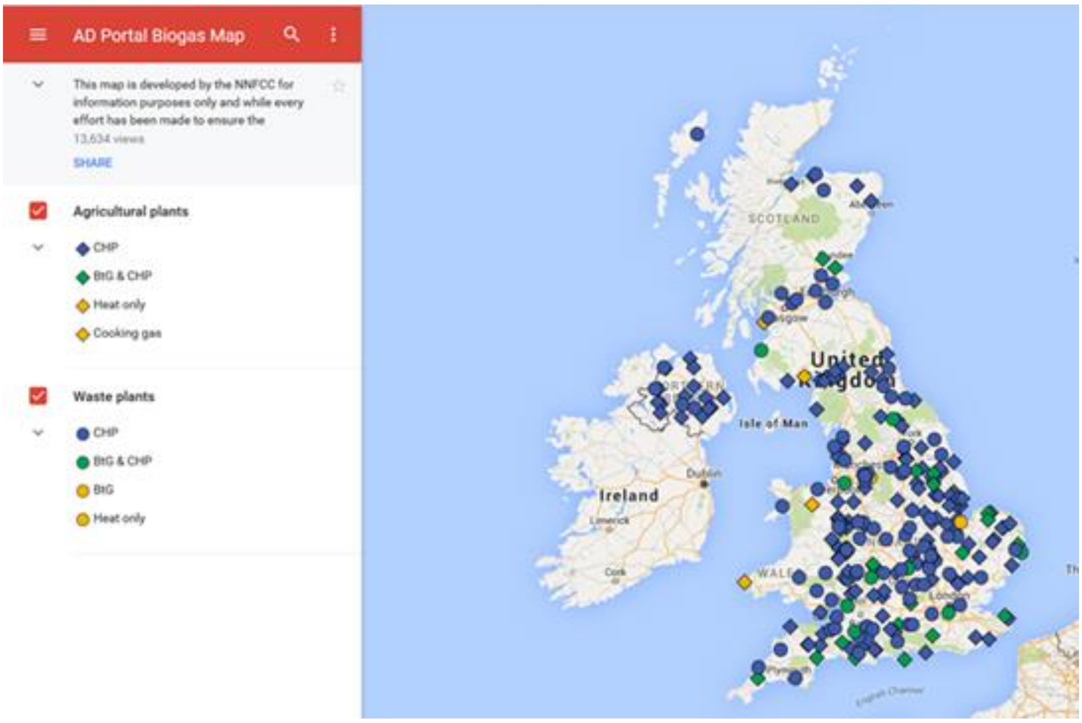

Figure 3. Distribution of Biogas plants in the UK using on-farm crops or domestic food waste as feedstock (Source: National Non-Food Crops Centre, 2015)

The UK drive to achieving a national Circular Economy with significant reduction in waste streams has resulted in the establishment of both commercial and on-farm anaerobic digestion (AD) operations. The use of AD as a means of energy generation from organic materials is an established technology that uses biomass crops and manures (on-farm) and industrial scale plants using food-wastes as feed-stocks, to generate methane. Residues (digestate) of the AD process are the spent liquids and solids remaining in AD vessels. This digestate has high elemental concentrations of essential plant nutrients, particularly nitrogen (N), and consequently has recognised potential for agronomic benefit. Its application to support agricultural production has co-benefits in that it substitutes for use of mineral fertilisers which have high economic, energy and environmental costs. Further, the residual organic fraction of digestate is in a persistent form suited to stabilise soil structure and to support the biological diversity intrinsic to sustainable soil function. The potential for use of digestate on agricultural land has not been realized (Riding et al., 2015). A lack of underpinning knowledge to guide best practice for digestate use has strongly hampered its widespread uptake (Insam et al., 2015). Although 66% of UK farms apply organic manure to at least one field (UK Gov, 2015), only 4% apply digestate. Emerging data has suggested that digestate has suppressive effects on soil-borne diseases by 20 to 90% (Campbell, 2006;

Wei et al., 2016). Thus an opportunity exists to ascertain the potential of digestate application as a component of FLN management.

Biological Control of FLN

Several microorganisms (estimated in few hundred of strains) have shown efficacy against soil-borne pathogens including nematodes. Some are the active substances of existing biopesticides. Those claiming to have a nematicidal effect include *Bacillus firmus* I-1582 and *Purpureocilium lilacinum* strain 251 (EU Pesticides database: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database-redirect/index_en.htm). Although several biological control agents have been previously developed, they rarely achieve a large market, due to competiveness with existing synthetic chemicals, which are cheaper, easier to apply, with a wide spectrum of activity and high efficacy. However, 91/414/EEC has now revived the interest in microbial biocontrol agents against soil-borne diseases in particular in integration with other agronomic practices or resistant/tolerant varieties (Velivelli et al., 2014). As with many of the novel methodologies being tested for nematode management the focus to date has been on PCN and RKN with minimal exploration of FLN. Potentially due to regulatory issues, the UK lags behind Europe in this area of pest management. In the context of FLN, the following are potentially pertinent:

Bacillus spp.

Members of the *Bacillus* (*B. subtilis*, *B. amyloliquefasciens*, *B. firmus* and *B. pumilus*) are among the beneficial bacteria mostly exploited as biopesticides to control plant diseases and nematodes. They are Gram positive rod-shaped bacteria that can form a protective endospore that can tolerate extreme environmental conditions.

In the rhizosphere, competition takes place for space at the root surface and for nutrients, noticeably those released as seed or root exudates. Competitive colonisation of the rhizosphere and successful establishment in the root zone is a prerequisite for effective biocontrol, regardless of the mechanism(s) involved. Direct antagonism involves the production of several microbial metabolites among which lipopeptides play the major role. Surfactin in particular is involved in the mechanism of resistance induction. The bio-nematicide *Bacillus firmus* also colonizes the rhizosphere of the plant where it parasitizes the eggs and larvae of nematodes especially RKN.

Bacillus strains are effective against a broad spectrum of plant pathogens and they can be used either as foliar application or root application before transplanting. The ability of the specific *Bacillus* strain to colonise and permanently establish on the roots is an imperative for soil or root application or efficacy is likely to be low. The advantage of *Bacillus* spp. is their long shelf-life at room temperature, given by the fact that they produce endospores.

Purpureocillium lilacinum

Purpureocillium lilacinum is a naturally occurring fungus commonly found in soils. Unlike many other *P. lilacinum* strains, the registered strain does not produce mycotoxins or paecilotoxins. It grows optimally at 21-27 °C, and does not grow or survive above 36 °C. The registered strain was isolated from infected nematode eggs. *P. lilacinum* parasitizes and subsequently kills eggs, juveniles, and adult females of various plant parasitic nematodes.

As a pesticide, *P. lilacinum* can be used to control plant feeding nematodes on many crops. It acts by infecting eggs, juveniles, and adult females of various plant pathogenic nematodes including *Meloidogyne* spp., *Heterodera* spp. and *Globodera* spp. and the FLN genus, *Pratylenchus*. It can be applied to agricultural soil through drip irrigation, or water in the suspension around base of each plant.

Arbuscular mycorrhizal fungi (AMF)

AMF form obligate mutualistic symbiotic associations with > 90% land plant groups, including carrot, improving plant nutrient uptake and mitigates against (a) biotic stress (Helgason et al., 1998; Jeffries et al., 2003). In arable soils they are diverse and AMF communities can be crop specific (Daniell et al., 2001; Davison et al., 2011). Also, AMF communities have been shown to change composition based on time within the growing season and plant host (Bennett et al., 2013). In a range of crop systems, the addition of AMF to soil has helped to manage nematodes (e.g. Affokpon et al., 2011). In a pot experiment, application of AMF spores compensated for yield loss in carrots caused by the FLN *Pratylenchus penetrans* and furthermore reduced the density of *P. penetrans* by 49% (Talavera et al., 2001). The ability to add spores at time of planting and to determine crop specific AMF communities for nematode management offers potential for exploration.

Threat of root knot nematode (Meloidogyne species)

As outlined at the beginning of this report, whilst this review has a sole focus on FLN, it would be a glaring omission not to refer to root knot nematode and the potential threat this nematode group poses to UK carrot and parsnip production. It would not be an exaggeration to state that c. 99% of all peer-reviewed publications consulted that referred to carrots and nematodes had a root knot nematode focus. Unlike the UK, long-term significant funding has been available to European research groups, primarily in Germany, Spain and the Netherlands, which has supported a diverse portfolio of research on the impact of root knot nematode on vegetable production (organic and conventional). Root knot nematodes have a widespread distribution through mainland Europe, but not the UK, with many of the species listed as quarantine organisms (Wesemael, et al., 2011). This aligns with RKN being recognised as the most economically damaging nematode group globally with a vast host range (Jones et al., 2013) with potential for devastating crop losses and major quality issues (Figure 4).

Figure 4. Typical symptoms of *Meloidogyne* species infection of carrots

It is therefore understandable that the nematological research focus in Europe is on RKN rather than FLN.

Concerning, however, is the anecdotal evidence during AHDB facilitated meetings with growers that RKN have been recorded associated with carrot production in the UK. It is not an understatement to say that if RKN becomes established in the UK, the impact of FLN will become insignificant to the industry. Recently it has been documented that *Meloidogyne minor*, a RKN species previously confined to amenity turf habitats in the UK (Fleming et al., 2008) has moved into agricultural land (Fleming et al., 2016). Thus growers should be alert to any infestation of RKN on carrot growing land and take sensible phytosanitary precautions not to spread nematodes or infected material from location to location. The one positive, is unlike FLN there is a significant body of research to learn knowledge and adapt and trial for UK conditions.

Recommended Research Areas

Based on the literature it is evident that the UK is significantly behind Europe and North America with regard to developing alternative management strategies for FLN. Consequently to ensure that carrot and parsnip production in the UK remains economically viable and sustainable, significant levels of research funding (or co-funding with other production sectors) will be required to adequately test and validate new methodologies under UK conditions (climate and soils). Whilst biological control is attractive, regulatory controls may in the short-term be a significant constraint. Therefore, nine key areas for research funding are identified as follows (six in bold are considered high priority):

i) Effective knowledge transfer to underpin the benefits of pre-plant soil testing for FLN

ii) Utility and efficacy of arbuscular mycorrhizal fungi for FLN management

iii) Understand the prevalence of RKN species in UK carrot/parsnip growing areas and maintain vigilance for RKN

iv) Can cover crops be integrated into a standard carrot/parsnip rotation for FLN management?

v) Field scale evaluation of biofumigant crops for FLN management

vi) Role of soil amendments to induce suppressive soils (potential focus on biochar and digestate)

vii) Determine the optimum organic matter type to mitigate FLN impact

viii) Ascertain whether changes in tillage regimes can significantly mitigate the impact of FLN on carrot and parsnip production

ix) Explore the utility of seed meal for management of FLN

Conclusions

In an UK context, this report has highlighted significant knowledge gaps regarding the impact and management of FLN related to UK carrot and parsnip production. Furthermore, current research funding in the UK for FLN related issues associated with root vegetable production is exceptionally poor compared to both other UK market sectors and the root vegetable industry in competing countries within mainland Europe. Thus, unequivocally the impact of the removal of approved nematicides (91/414/EEC) has the potential to have a proportionately greater impact for the UK carrot/parsnip industry than European rivals. Whilst there is an active BBSRC-HAPI funded project evaluating biofumigation as a sustainable alternative to pesticides, including FLN on carrots, final analysis will not be immediately available.

Key issues raised during face to face meetings with growers and agronomists were uncertainty on the tangible impact of FLN on carrot and parsnip production with some members of the industry convinced that FLN had a significant yield impact on their crops whereas other sector members remained ambivalent. Furthermore, there was a similar split amongst practitioners regarding the value of pre-plant soil testing for FLN with a number of growers and agronomists regularly submitting soils for testing whilst others considered there was no economic benefit to their business to have soils tested. A consensus amongst practitioners was that lack of available land was a significant constraint to increasing the length of the rotation.

Therefore in the short-term, options for growers to better manage the impact of FLN on carrots and parsnips appear limited. However, immediate options that could be considered are:

- i) Where possible increase length of rotation, even one year may be beneficial
- ii) Ensure that rotation crops include those that are poor hosts of FLN that impact carrot and parsnips thus limit, where possible, sugar beet, peas, beans and potatoes in the rotation
- iii) Monitor FLN by soil testing using where possible molecular diagnostics to minimise costs and improve specificity of testing
- iv) Apply organic matter prior to planting
- v) Where land is owned, rather than rented, consider inclusion of a cover crop within the standard rotation
- vi) Be alert to the presence of root-knot nematode and take appropriate measures to minimise spread

Knowledge and Technology Transfer

In terms of knowledge transfer, the author presented an invited talk at the British Carrot Growers Association AGM on FLN. Furthermore, AHDB facilitated two meetings with carrot producers during the contract period where frank discussion was held with all parties which have been translated into recommendations of this report. As per the terms of FV 447, a fact sheet for growers post report submission will be developed. An article for Grower will be produced at the conclusion of the project.

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Appendix

Appendix 1. List of laboratories that are known to provide a FLN diagnostic service

ADAS High Mowthorpe:

http://www.adas.uk/Services/Services-Categories/category/agricultural-advice-1

James Hutton Limited: http://huttonltd.com/analytical-services.aspx

Eurofins:

http://www.eurofins.com/agroscience-services/chemistry/plant-disease-diagnostics/

Fera: Plant Clinic: http://fera.co.uk/plantClinic/priceLists/potatoesPrice.cfm

NIAB: http://www.niab.com/pages/id/141/Seed_Pathology http://www.niab.com/labtest/

SRUC: http://www.sruc.ac.uk/info/120118/crop_clinic

Appendix 2. Summary of SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis of FLN impact on sustainable production of carrot and parsnip in the UK

Strengths

Underpinning FLN expertise in the UK

Agile and knowledgeable grower base

Good research infrastructure to conduct FLN research

Engagement of research and grower communities

Weaknesses

Historical apathy towards FLN

Assumption by growers that all nematodes are the same

Comparative lack of research funding compared to other nematode groups e.g. PCN

Lack of available land to increase length of rotation

Much of the land used for carrot and parsnip land is rented and not in grower control

Frequent use of poor rotational crops i.e. good hosts for FLN

Lack of transparency regarding previous cropping and FLN status of rented land

Degree of distrust by certain growers towards soil testing for FLN

Historical use of pot based experiments

Opportunities

Development of sustainable strategies for long-term carrot and parsnip production

Multi-layered IPM strategies

Cutting edge research

Field-scale experiments rather than pot based

Collaborative research with global research groups – learn from other crop systems

Threats

Presence and subsequent spread of root knot nematode species in UK carrot and parsnip growing areas

Lack of agronomic knowledge on how to deal with UK root knot nematode infestations

Aging FLN community within the UK that requires strategic successional planning

Changing political landscape leading to potential exclusion of UK research community from European IPM research networks

UK research funding diverted to other sectors as a consequence of current politics

