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Plant Parasitic Nematodes

INTRODUCTION

Plant parasitic nematodes are microscopic in size, with the most abundant species typically being 1-1.5mm long. In general they can be classified as either being migratory or sedentary. Nematodes damaging to potatoes occur in both of these groupings:

- Migratory ectoparasites. These remain in the soil and feed from root cells, moving between the roots of different host plants (e.g., stubby root nematodes and needle nematodes)
- Migratory endoparasites. These spend time both in soil and within their host plants. They can enter plants either via roots or haulm, the latter at the interface with the soil surface, and on egress create a wound site that can be infected by bacteria or fungi leading to a range of diseases. This is most widely reported for root lesion nematodes.
- Sedentary endoparasites. In these species the female nematodes lose mobility once they are inside the host roots. In the case of potato cyst nematodes, the females swell with eggs and are visible as cysts protruding from the root. Root knot nematodes are also endoparasites. In these species the females don't form cysts, instead eggs are laid into an egg sac and both the female and the egg sac tend to be buried in galls induced by nematode feeding. When infected plants are lifted the galls may be visible on the roots.

In this document we will refer to all the plant parasitic nematodes that aren't sedentary endoparasites as free living nematodes (FLN). The information provided will mainly be restricted to the FLN that are damaging to potatoes. Therefore, a much wider range of FLN species occur than are listed here.

DISTRIBUTION OF FLN

Because of their small size FLN are unable to move rapidly through soil under their own motion and, except in coarse sandy soil, they are confined to the spaces between soil aggregates. Therefore, their activity and movement are affected by soil structure, aeration and moisture[1]. For example, fine particle size and close packing as in dense clays are unfavourable to stubby root nematodes. Typically FLN do not move over large distances laterally (e.g., approximately 0.5 m per year) but they can be dispersed longer distances by equipment, tillage, runoff and erosion. Within individual fields FLN have an aggregated distribution.

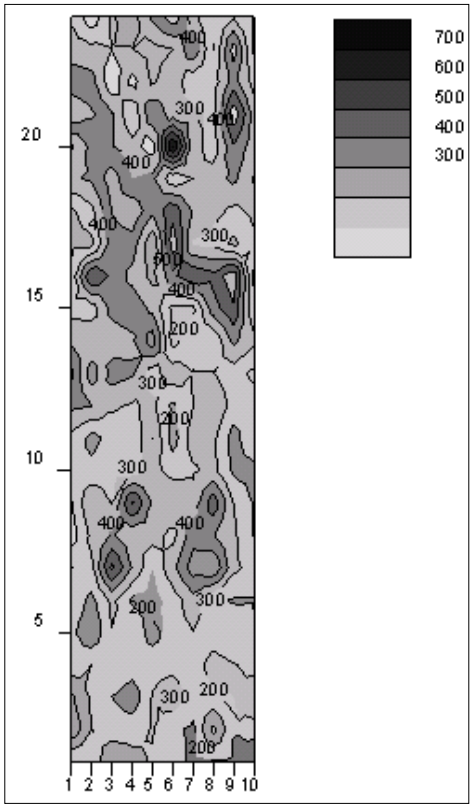

Figure 1 (left) shows the numbers of stubby root nematodes recorded within a 240 x 50 m potato field trial in GB. Numbers (indicated by grey to black shading) are expressed as numbers of nematodes per 200 g of soil.

In general, FLN show a vertical distribution through the soil e.g., stubby root nematodes move vertically in the soil profile by up to 90 cm, mostly following the water table. The distribution of root lesion nematodes, such as *Pratylenchus penetrans*, can be closely related to the presence of host roots[2].

GENERAL SYMPTOMS

Direct feeding by nematodes can drastically decrease a plant's uptake of nutrients and water. When crops show an in-field patchy decline, lack of vigour, chlorosis or slower than normal growth nematodes may be the cause. During periods of stress or where there are nutrient deficiencies, nematode-infested plants will tend to be affected first. Other symptoms of FLN attack include a crooked or bushy appearance of tap roots, fleshy tap roots, stunted, stubby small root systems with excessive branching; small roots which are large near the tip; sparse lateral roots; brownish to black spots or streaks or discoloured necrotic areas on the roots.

There is no currently known varietal resistance in potatoes to direct feeding damage caused by FLN. This is likely to be due to the nature of their feeding behaviour. The majority of species are migratory ectoparasites which remain outside the root and feed from the outside with rapid probing movements.

FLN AS VIRUS VECTORS

Certain species of stubby root nematodes transmit Tobacco Rattle Virus (TRV) when they feed on potatoes. These are referred to as virus vectors. Less than 10% of all the stubby root species are known to be virus vectors. The virus is transmitted when the nematodes feed on daughter potato tubers at tuber initiation. It is also proposed that late season TRV transmission can occur at the onset of senescence, though this has to yet be determined experimentally. The virus can cause necrotic arcs in the tuber flesh (corky ringspot or spraing) and stem-mottle (distortion, stunting and mottling) and aucuba (yellow spots) in the foliage. Spraing symptoms can also be the result of infection by a different virus, Potato Mop Top Virus (PMTV), which is transmitted by the powdery scab pathogen, *Spongospora subterranea*. TRV-induced spraing is visually indistinguishable from PMTV-induced spraing although there are diagnostic assays available which can differentiate the virus present. Both viruses may be present in a single tuber.

TRV and PMTV symptoms can be confused with some other tuber problems[3]. For example, physiological disorders, in particular internal rust spot, can cause symptoms similar to spraing. Both TRV and PMTV can also induce external necrotic rings on tubers, which are sometimes seen without any internal symptoms. As a result, it is possible (especially in non-typical cases)

to confuse these symptoms with potato tuber necrotic ring disease caused by the tuber necrosis strain of Potato Virus Y (PVY-NTN)

TRV

TRV has a wide host range including many arable weed species, including field pansy, knotgrass, groundsel, shepherd's purse and chickweed. The plants don't usually become systemically infected, instead the virus is mostly restricted to the roots. Those weeds that do become systemically infected (e.g., chickweed) often don't show any foliar symptoms. TRV can over-winter in perennial weed species and in a few cases can be transmitted through weed seeds (e.g., field pansy) in the absence of nematodes. During a study[4] of the roots of weeds present within a developing barley crop the following proportion of the weeds tested positive for the presence of TRV:

Common name	**Species name**	**Number of plants shown to be positive for TRV/number of plants of that weed species tested**
Chickweed	*Stellaria media*	10/11
Fathen	*Chenopodium album*	3/14
Mayweed	*Matricaria discoidea*	2/3
Groundsel	*Senecio vulgaris*	1/3
Small nettle	*Urtica urens*	0/6
Hemp nettle	*Galleopsis* species	0/3

The researchers concluded that some weeds are more susceptible to TRV than others.

BIOLOGY OF IMPORTANT FLN

TRICHODORUS AND *PARATRICHODORUS* SPECIES (STUBBY ROOT NEMATODES)

They are collectively referred to as trichodorids and their feeding results in thickened, stubby roots which provides the common name for the group. They are migratory ectoparasites. In UK, the conditions that favour these FLN are sandy loams or loamy sand soil with a sand fraction between 80-90% and a silt fraction of less than 10%. There are six life stages: the egg, four juvenile stages (J1, J2, J3 and J4) and the adult stage. Generally, under field conditions trichodorids reproduce from spring to autumn. The temperature range for reproduction is 15 to 30°C. *T. primitivus* can complete their lifecycle in ~22 days at 22°C. Soil temperatures were recorded at 20cm depth in potato ridges from the start until the finish of the growing season at different sites around the UK during 2010-2012[5]. The average temperatures at that depth were ~11 to 17°C.

Globally, the species that are known vectors of TRV are:

Trichodorus primitivus
Trichodorus similis
Trichodorus viruliferous
Trichodorus cylindricus
Paratrichodorus allius

Paratrichodorus anemones
Paratrichodorus hispanus
Paratrichodorus pachydermus
Paratrichodorus teres
Paratrichodorus tunisiensis
Nanidorus nanus (previously referred to as Paratrichodorus nanus)
Nanidorus minor (previously referred to as Paratrichodorus minor)

The species considered most relevant to UK agriculture are highlighted in bold. *P. anemones* has a relatively restricted distribution within the Vale of York & Lancaster areas.

Tobacco Rattle Virus (TRV) exists as different isolates which can be categorised as either Multiplying (M) or Non-multiplying (NM) types. The latter lack a part of the viral structure (coat protein) that occurs in the M-type. In NM-type infections the virus still replicates and spreads from cell to cell within the plant and it can result in symptoms (spraing). However, the absence of the genetic information that leads to the production of the coat protein ultimately means that NM-types of TRV cannot be transmitted by the vector nematodes from plant to plant. Therefore they presumably initially arise as a result of transmission of M-type virus by stubby root nematodes, with the virus subsequently losing the genetic information needed to produce the coat protein once it has infected the plant.

Antibody-based techniques were developed in the ~1980s which rely on the presence of the coat protein to detect and differentiate between isolates. Therefore these techniques provide information on M-type infections only. Isolates that have been differentiated using the antibody-based techniques are referred to as serotypes. In the UK, the stubby root nematodes considered important vectors of TRV are *T. primitivus*, *T. similis*, *P. anemones* and *P. pachydermus*. In general, *Trichodorus* and *Paratrichodorus* species transmit the RQ and PRN serotypes of TRV, respectively.

From a management perspective there is currently limited practical benefit of knowing which serotype is present and there are no commercial tests offered that provide this information. It has been reported by agronomists that some varieties don't always perform as expected from their TRV spraing resistance rating. This is attributed to the TRV serotype present in the field being different from the serotype where the resistance testing was carried out. This hasn't been studied experimentally in any detail. Both serotypes can co-exist in fields. As the antibody-based techniques mentioned above only detect M-type virus it would be possible to have tubers which show spraing symptoms but where TRV is not detected by the antibodies. Molecular assays have been developed which can detect both the M and NM-types of TRV.

On the whole, nematodes become viruliferous after being in the presence of infected hosts for fewer than 24 hours and the actual feeding times required for different viruses range from 15 to 60 minutes. In the case of TRV, virus particles are retained on the lining of the nematode pharynx and the virus is lost at each moult. Typically FLN will moult 4 times during their lifecycle. The virus does not pass through the egg stage.

PRATYLENCHUS SPECIES (ROOT LESION NEMATODES)

These are migratory endoparasites. They indirectly cause plant tissue necrosis as a consequence of their migration and feeding. Ten species have been recorded from soils outdoors in the UK and the species present vary with soil type. Those most commonly associated with agricultural soils are:

Pratylenchus penetrans
Pratylenchus crenatus
Pratylenchus fallax

Pratylenchus neglectus
Pratylenchus thornei

P. penetrans is currently considered the species of most relevance to potatoes in UK. However, because of the difficulties for non-specialists of distinguishing between *Pratylenchus* species using microscopy/visual identification root lesion nematodes were often grouped as *Pratylenchus* species. This may have underestimated the importance of species other than *P. penetrans*. More recently molecular diagnostic assays have been reported which identify individual species (e.g., *P. penetrans*; *P. thornei)*. A PhD student supported by AHDB has been investigating the prevalence of lesion nematodes in GB.

Despite being parasitic within plant roots, *Pratylenchus* species have simple lifecycles. All lifestages of *Pratylenchus* species can occur in a parasitized host. The duration of the lifecycle may vary from 1-2 months depending on environmental conditions. Between successive crops the nematodes may overwinter in root remnants. They also have a wide host range including weed species.

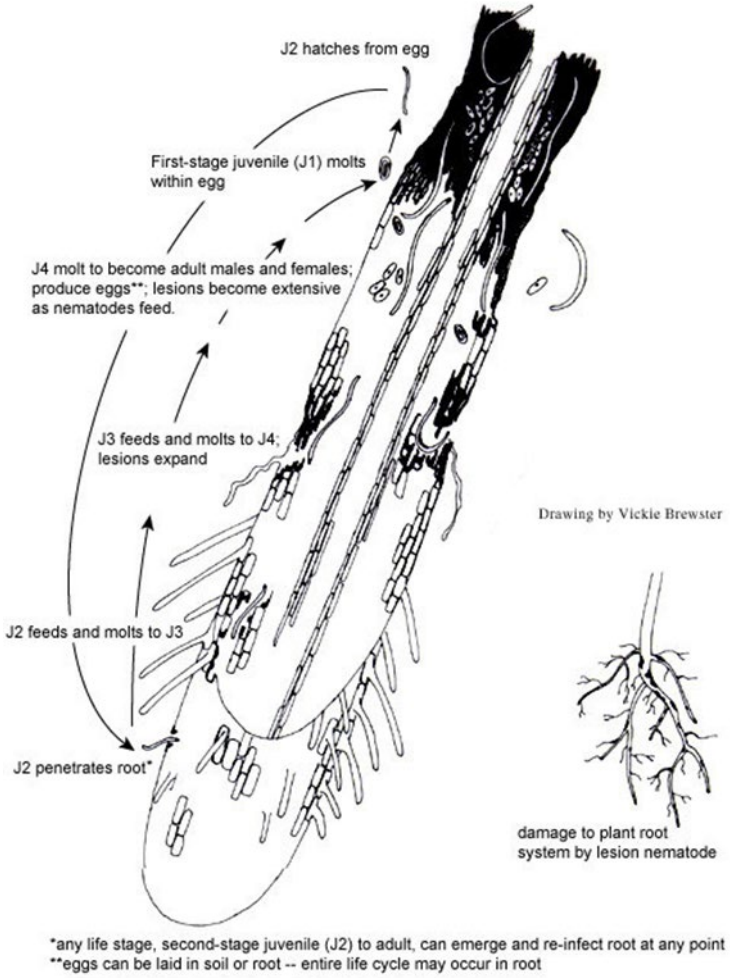

Figure 2. Lesion nematode lifecycle from APSNet.org website 2015.

Lesion nematode feeding causes damage which is a site for secondary infection by bacteria and fungi (e.g., *Verticillium dahliae*). Information from the USA[6] describes how early dying caused by *V. dahliae* is aggravated by populations of *P. penetrans*. The same nematode has been linked to common scab-like symptoms in studies in Norway[7]. Results from a study of 8 potato fields in Sweden indicated that there was an interaction between stubby root nematode

or potato cyst nematode and stem canker but not between *Pratylenchus* species and *Rhizoctonia solani*[8].

LONGIDORUS SPECIES (NEEDLE NEMATODES)

These are migratory ectoparasites. They feed on a wide range of woody and herbaceous plants, attacking the root tips. There is little published information on yield losses in potatoes directly attributable to their feeding. There is a suggestion that needle nematodes can affect emergence of potatoes when emergence is slow, due to planting into cold soil conditions. However, there is no clear evidence, and there may be a link with plant-pathogenic diseases accessing sprouts via nematode feeding lesions. Direct damage is observed in root crops such as carrot, parsnip and sugar beet (e.g., fanging, docking disorder). *Longidorus elongatus* is prevalent in lighter soils in Scotland and NE England, while *Longidorus macrosoma* is found on heavier soils in southern England.

DITYLENCHUS DESTRUCTOR (POTATO TUBER ROT NEMATODE)

It is a migratory endoparasite and a rare problem in the UK. Symptoms include conical pits with skin splitting. *Ditylenchus dipsaci* can also infest potato tubers.

MANAGEMENT

Soil sampling for FLN is highly recommended to support decisions about field selection. The page at the end of this document describes a standard soil sampling protocol for FLN. This has been derived from the recommendations provided by laboratories offering a FLN soil testing service. A list of the laboratories is provided.

Note: as *Pratylenchus* species spend time within the host root as well as in soil, an accurate estimate of their density requires that nematodes are extracted from both the root and soil. Practically, sampling is effectively as per other FLN i.e. collection of soil cores. However, reliance on soil samples alone is likely to provide an underestimate of the numbers of *Pratylenchus* present. Researchers in USA reported that ~50% of the total population of *P. penetrans* was found in the root fraction after harvest of potatoes[9].

DAMAGE THRESHOLDS FOR FLN DIRECT FEEDING DAMAGE

There is limited published information on the relationship between the numbers of FLN present and yield loss or reductions in quality of potatoes. The reports that are available refer to:

- Damage thresholds for *Trichodorus* being at/above100 nematodes in 250 g soil.
- Damage thresholds for *Longidorus* being 20-25 nematodes/250 g soil.
- Damage thresholds for *Pratylenchus penetrans* ranging from 25 nematodes/250 g soil up to 625 nematodes/250 g soil.

All the studies are more than 10 years old and may not be relevant to current UK potato varieties. A research project[10] initiated in 2011 studied the performance of 12 varieties in replicated plot trials at four sites in each of three years. The FLN counts reported from individual plots ranged from 1-870 trichodorids per 200 g soil and 0-141 *Pratylenchus* species per 200 g soil. Plots were either treated with nematicide (Vydate 10G) or left untreated. At a given site/year the trial design required that all varieties were harvested at the same time. Across the years and sites there were a range of conditions at planting/crop emergence. These impacted on the rate of crop emergence and it was not possible to identify an effect of FLN numbers on marketable yield across all sites/years. For some varieties, the data suggest that crops with long development time (measured as days to 50 % ground cover) show greater

benefit from Vydate 10G under high trichodorid numbers. The varieties that showed this effect especially strongly were: Shelford, Crisps4All, Melody.

The other varieties included in the trials (and which didn't show an especially strong effect on marketable yield) were:

Maris Piper
Pentland Dell
Shepody
Markies
Innovator
Lady Rosetta
Casablanca
Saxon
Harmony

Therefore, there do not seem to be simple damage thresholds which can be considered applicable to all varieties in all situations.

TRV SPRAING AND VARIETY CHOICE

Unlike direct damage, the risk of TRV infection is unrelated to FLN numbers with a single nematode representing as high a risk as multiple hundreds of FLN. This is because the efficiency of virus transmission is important. If stubby root nematodes are detected in a soil sample it is recommended that tests are carried out to determine if the nematodes are carrying TRV. This can be done via bait tests in which bait plants are grown in a sample of the soil. Visual and/or molecular methods are used to determine if TRV has been transmitted to the bait plant (indicating that at least some of the trichodorids in the sample are carrying the virus).

Varieties differ in their response to TRV infection and have been categorised[11] as follows:

- **Resistant**: the varieties do not show any symptoms and virus particles cannot be detected in the plant, including in the tubers. However, it is possible that some isolates of TRV have arisen that can overcome this resistance.
- **Spraing sensitive:** varieties which exhibit spraing symptoms in the tubers and surface lesions and malformations. Virus particles are rarely found in the plants, including the tubers.
- **TRV susceptible:** varieties which show few if any symptoms in the tuber flesh but become systemically infected so that virus particles can be detected throughout the plant. After several generations such potato plants produce smaller and more irregular tubers. There may also be effects on quality (e.g., after cooking blackening)[12]. Infected tubers can act as a reservoir of TRV and result in the movement of the virus to new sites in which the trichodorids might have previously been TRV-free but which will subsequently acquire the virus after feeding on the roots of the infected plants.

For some varieties a resistance rating (on 1-9 scale) is reported by NIAB TAG. The ratings are based on the incidence of symptoms in field trials on land infested with viruliferous (TRV) nematodes.

Table 1. Varieties categorised as resistant, spraing sensitive or TRV susceptible by researchers at the James Hutton Institute (formerly SCRI) based on the results from field trials. NIAB TAG rating provided in brackets.

Resistant	**Spraing sensitive**	**TRV susceptible**
Record (9)	Pentland Dell (1)	King Edward (6)
Saturna (7)	Maris Bard (2)	Sante (6)
Resistant	**Spraing sensitive**	**TRV susceptible**

Nicola (8)	Picasso (1)	Nadine (6)
Lady Rosetta (8)	Russet Burbank (1)	Shepody (6)
Fianna (8)		Saxon (7)
		Marfona (6)
		Rocket (5)

Figure 3. Examples of TRV spraing symptoms in cut tubers (a) Maris Peer and (b) an unidentified variety:

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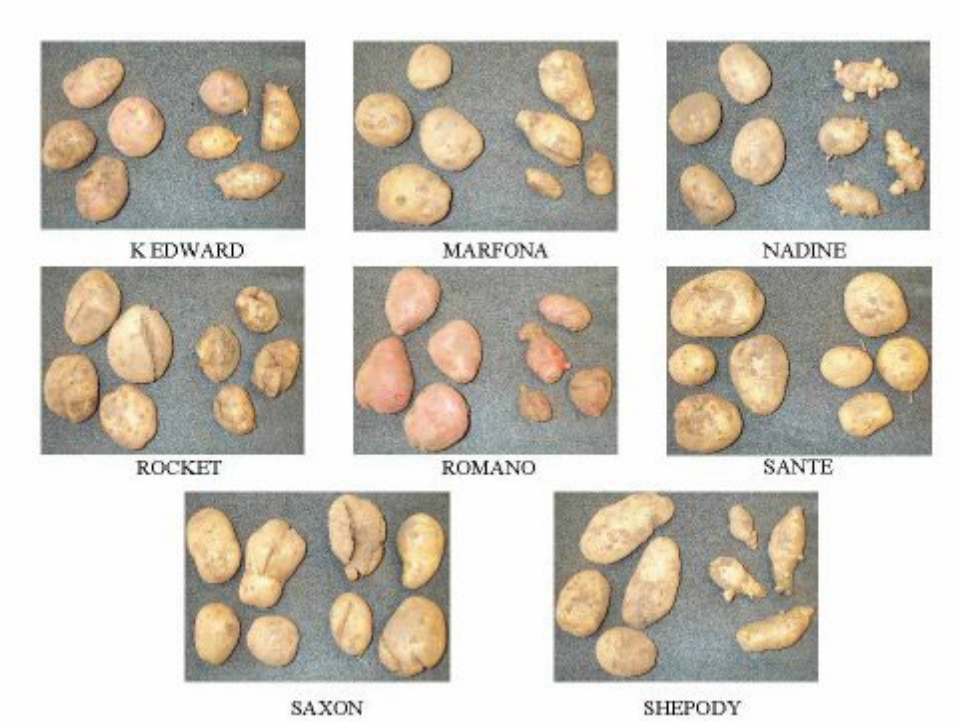
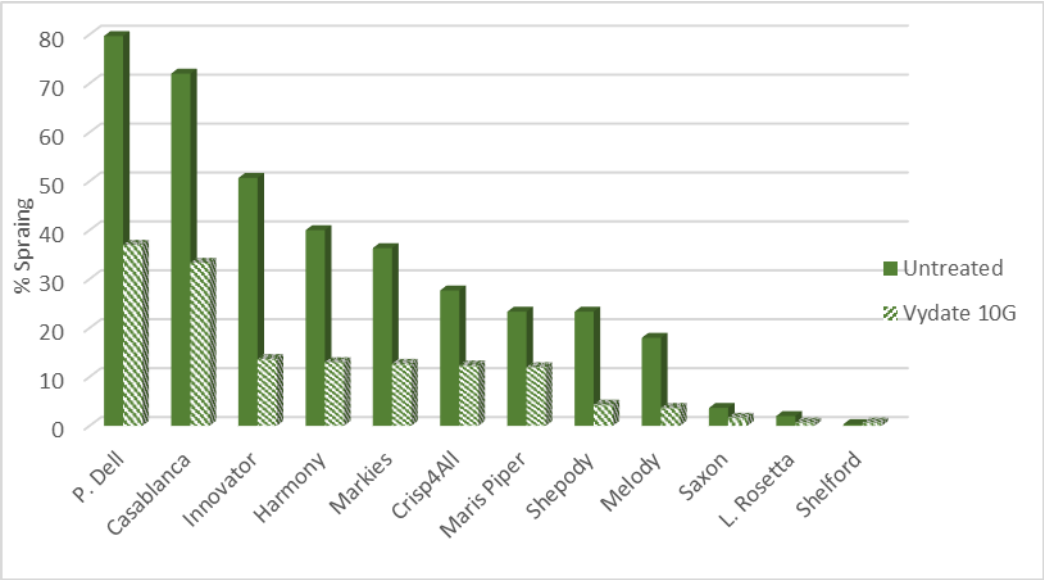
Figure 4 (below). Comparison of harvested tubers of susceptible cultivars: healthy tubers on left and TRV-systemically infected tubers on the right of each photo.



In the research project initiated in 2011, two of the sites in each year were known to have TRV present. Twelve varieties were grown and the tubers were assessed for spraing. Diagnostic assays were used to confirm the presence of TRV. Results from one of the sites is shown. The

site was chosen knowing that previously there had been high levels of spraing recorded and it would not have been chosen for commercial production of a TRV sensitive variety but it was useful as a trial site.

Figure 5. The mean % of tubers exhibiting spraing symptoms*. Results from a site in Shropshire (2012). 50 cut tuber samples per replicate were assessed for spraing symptoms. The presence of TRV in samples of infected tubers was confirmed using diagnostic tests performed by SASA.



*Data refers to the presence or absence of necrotic arcs in the tuber flesh. Other impacts of FLN damage, such as 'spots and marks', are not included in the figures.

NEMATICIDES

Granular nematicides allow the crop to grow through the most susceptible period at tuber initiation by preventing or reducing nematode movement near the root area.

Table 2. List of nematicides with relevant information (as specified in product labels).

Active Ingredient (Product)	Control	Application	Harvest Interval
Fosthiazate (Nemathorin 10G)	A contact nematicide/insecticide for the control of the potato cyst nematode (*Globodera rostochiensis* and *Globodera pallida*) and a reduction of potato tuber damage caused by wireworm and reduction of spraing transmitted by free-living nematodes.	Broadcast overall application 30 kg product/ha. See label for detailed advice on incorporation.	Do not desiccate or harvest crops for human or animal consumption for at least 17 weeks after application.
Fluopyram Velum Prime	Velum Prime can be used for the suppression of plant parasitic nematodes	Broadcast or in-furrow in 200–500 litres of water per hectare. See label for details of nozzles for in-furrow application and incorporation for broadcast application	None

WEED/VOLUNTEER CONTROL

As many weeds are hosts of TRV, good broad-leaved weed control should reduce the reservoir of virus in the field. This is important as the trichodorids will lose the virus each time they moult. Some plants are non-hosts for TRV[13]. If they are a good host for trichodorids it is expected that the nematodes will feed on them and transmit the virus, but that this will not be multiplied in the plant, therefore reducing the reservoir of virus in the field. Good weed/volunteer control is important to ensure that weeds don't act as a source from which the nematodes can be become reinfected with TRV. Barley is not a good host for TRV. However, it may increase trichodorid numbers. Oilseed radish is also considered not a good host for TRV.

BIOFUMIGATION

'Biofumigation' refers to the release of compounds (principally isothiocynates) when certain brassica crops are chopped/incorporated into soil. In GB, most research activity has focussed on the use of biofumigant crops for potato cyst nematode management. The research has focussed on crops such as Indian mustard (*Brassica juncea*) and oilseed radish (*Raphanus sativus*). These have been drilled in summer months (June-August) and grown for a period of 10-14 weeks. The crops receive N and S inputs and are grown so as to maximise biomass production. It is recommended that incorporation takes place at mid-flowering. Following chopping the brassica residues need to be incorporated in quick succession. The efficacy of biofumigation is considered to be influenced by factors such as soil pH and soil moisture at the time of incorporation. There is less information on the impact of biofumigants on FLN. The research project initiated in 2011 evaluated the effect of control options on TRV spraing. The treatments studied included the growing of an oilseed radish or Indian mustard crop prior to potatoes. The follow-on potato crops were then either treated with a nematicide (Vydate 10G) or left untreated. Only the application of Vydate 10G (55kg/ha broadcast and incorporated) produced a consistent positive effect reducing spraing levels in the susceptible variety Pentland Dell. Other researchers have reported higher numbers of root lesion nematodes after incorporation of various plant species, including biofumigants, compared to fallow plots on a sandy soil in the Netherlands. Other studies have evaluated the effect of cover crops and/or green manures on FLN numbers. A study of FLN affecting carrots[14] indicated that stubby-root nematodes were significantly increased in plots where a green manure (Sudangrass) had been drilled in autumn, eventhough the plants were defoliated by frosts. The Best4Soil website provides information on the host status and damage sensitivity of crops for some nematode species.

SUMMARY

- Soil sampling for FLN is highly recommended to support decisions about field selection.
- If the field history isn't known (i.e., previous records of spraing occurring) and stubby root nematodes are detected in a soil sample, it is recommended that tests are carried out to determine if the nematodes are carrying TRV
- There is a poor relationship between stubby root nematode numbers and the risk of TRV spraing. Low numbers of nematodes can represent as high a risk as hundreds of nematodes.
- Potato varieties vary in their response to TRV infection
    - Resistant varieties: do not show spraing symptoms
    - Spraing sensitive varieties: show spraing symptoms
    - TRV susceptible varieties: show few spraing symptoms in the tubers but the virus occurs throughout the plant, passes to daughter tubers and after several generations the plants produce smaller, more irregular tubers. Tuber quality can also be adversely affected.

- Resistance ratings are available for some varieties. These are based on the incidence of spraing symptoms in field trials on land infested with stubby root nematodes known to carry TRV.
- Weeds can be hosts of TRV and act as reservoirs of the virus.
- Some plants are not hosts for TRV (e.g., barley) but they may be good hosts for the nematodes and increase their numbers.
- The length of the rotation between potato crops is of limited importance for TRV management.
- TRV susceptible varieties (which show few symptoms but TRV occurs throughout the plant) can be a route to contaminate land currently free of TRV. If land is known to be free of TRV choose the variety/ source of seed potatoes with care so as not to introduce TRV onto the land.

ACKNOWLEDGEMENTS

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LITERATURE SOURCES

Several sources of information were used to produce the summary:

1: Jones and Jones. Pests of Field Crops. 1984
2: Vertical distribution of *P. penetrans* under four field crops. MP Pudsaini *et al.* Nematology. 2006.
3: Detection of potato mop top virus and tobacco rattle virus using a multiplex real-time fluorescent reverse-transcription polymerase chain reaction assay. RA Mumford *et al.* Phytopathology. 2000.
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5: Population dynamics of potato cyst nematodes in relation to temperature. VC Blok *et al.* AHDB Potatoes Report R433. 2015.
6: *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Idaho. MJ Martin *et al.* Phytopathology 72: 640-644. 1982.
7: Field damage in potato by lesion nematode *Pratylenchus penetrans*, its association with tuber symptoms and its survival in storage. R. Holgado *et al*. Nematologia Mediterranea 37(1) 2009.
8: Interactions between some plant parasitic nematodes and *Rhizoctonia solani* in potato fields. P Björsell. Master’s Thesis. Swedish University of Agricultural Sciences. Uppsala 2015.
9: Importance of depth in soil, presence of host roots and role of eggs as compared to vermiform stages in overwintering of *P. penetrans.* RA Dunn. J. Nematology 4:221-222. 1972.
10: Strategies for quantifying and controlling free living nematode populations and consequent damage by TRV to improve potato yield and quality. 2011-2016. Funded by Innovate UK & AHDB Potatoes.
11 & 12: R276 Free living nematodes and spraing. British Potato Council Research Review. 2006.
13: Trichodorid vectors of serologically distinguishable strains of tobacco rattle tobravirus occurring in Germany and the use of antagonistic plants to suppress spraing disease in potato. C Petz. Dissertation zur Erlangung des Doktorgrades des Fachbereichs Biologie der Universität Hamburg. 2003.
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SOIL SAMPLING FOR FREE LIVING NEMATODES

The general guidance provided below is summarised from information available from several of the laboratories which process samples to detect and quantify FLN populations.

Background: within fields FLN have an aggregated distribution and they will also move vertically in the soil profile e.g., trichodorids have been reported to move vertically by up to 90cm

Timing of sampling: from October until March (inclusive). Sampling during extremely cold, frosty, wet or dry conditions is not recommended.

Sampling area: Subdivide fields into units no greater than 10 acres (4 ha). 1 ha units are preferred and they will provide more accurate results than samples taken from 4ha units. Be aware of differences in soil type and previous cropping history and sub-divide fields into units to take account of these.

Sample collection: The majority of laboratories recommend sampling at random points using a W-shaped sampling pattern in each unit. Use a narrow-bladed trowel or "cheesecorer". Some laboratories indicate the minimum dimensions required e.g., take samples at least (2.5 cm) across and (20-25 cm) deep. A minimum of 50-60 cores per 1ha is preferred, sufficient to provide 1kg of soil. Take more cores if there is less than 1kg. Each 1kg sample should be sealed in a strong polythene bag. Record/attach details relevant to the sample on the bag.

Clean the sampling implement ("cheesecorer" or trowel) between different units (e.g., 1 ha blocks).

Laboratories offering FLN soil testing:

- ADAS High Mowthorpe: Tel: 01944 738646.
- James Hutton Institute. https://www.huttonltd.com/case-studies/free-living-nematode-diagnostic
- Fera: https://www.fera.co.uk/catalogsearch/result/?q=Nematodes
- NIAB: https://www.niab.com/services/laboratory-labtest/niab-labtest/pathogen-diagnostics
- SRUC. https://www.sruc.ac.uk/info/120118/crop_clinic/509/sampling_guide/3

TRV testing

Tests are available which provide information on whether TRV is present. In some cases this involves a bait test in which bait plants are grown in a sample of the soil. Visual and/or molecular methods are used to determine if TRV has been transmitted to the bait plant (indicating that at least some of the trichodorids in the sample are carrying TRV). An alternative method involves the use of molecular techniques to determine if nematodes extracted from a soil sample are carrying TRV (no bait plant is involved).

Laboratories offering TRV testing:

- Fera: https://www.fera.co.uk/spraing-test-for-soil-trv.html
- James Hutton Institute: roy.neilson@hutton.ac.uk
- NIAB: https://www.niab.com/labtest/