FREE LIVING NEMATODES- RISKS FOR POTATOES

(SUMMARY OF INFORMATION FROM WORK PACKAGE 2 TRIALS)

Plant parasitic free living nematodes (FLN) pose a risk to potatoes via two routes:

(i) Direct feeding damage to plant roots.

(ii) Transmission of Tobacco Rattle Virus (TRV) which can cause spraing in some varieties.

It is difficult to accurately predict the risk of either of these damaging effects occurring. This document summarises the factors to be aware of when considering the potential for effects on marketable yield to occur. It includes examples from the field trials (work package 2 of the research project[1]) on how the factors can combine to generate different levels of risk.

INTRODUCTION

The biology of FLN is discussed in detail in other publications (e.g., Plant Parasitic Nematodes) and only a summary is provided here. In general, they have one of two different lifestyles: some (e.g., stubby root nematodes [*Trichodorus* and *Paratrichodorus* species] and needle nematodes [*Longidorus* species]) remain in the soil and feed from root cells, moving between the roots of different host plants. In addition to causing direct feeding damage, some (but not all) stubby root nematodes can transmit Tobacco Rattle Virus (one of the causes of 'spraing' in potatoes) between plants. The stubby root nematodes (collectively referred to as trichodorids) of importance in UK include *Trichodorus primitivus, Trichodorus similis*, *Paratrichodorus primitivus* and *Paratrichodorus pachydermus.*

Other nematodes that are often categorised as FLN (root lesion nematodes [*Pratylenchus* species]) spend time both in soil and within their host plants. The entry/exit wounds they cause on roots can subsequently be infected by pathogens leading to a range of diseases.

Tobacco rattle virus (TRV) is able to infect more than 400 plant species including some arable weeds such as Common Chickweed and Shepherd's Purse. These can act as a reservoir of the virus in the field. The virus can also infect sugar beet and bulbs (gladiolus, narcissus, tulip) as well as potatoes.

Direct feeding by FLN can drastically decrease a plant's uptake of nutrients and water. When crops show an in-field patchy emergence, 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 in potatoes include poorly developed root systems often with thickened stunted roots; small roots which are large near the tip; sparse lateral roots; brownish to black spots, streaks or discoloured necrotic areas on the roots. Reports from agronomists indicate that emerging stems and stolons can be affected by similar brown discolouration and often this is associated with twisting below the soil surface. These symptoms can be easily confused with *Rhizoctonia* damage, which is often present within the same plant and/or field.

TRV can cause necrotic arcs in the tuber flesh (spraing or corky ringspot) which render crops unsuitable for marketing. Other symptoms, which depend on variety and environmental conditions, can include 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 *Spongospora subterranea,* the cause of powdery scab. 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. 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 $^{(PVY\text{-}NTN)}$. Given this, it is important that the precise cause of symptoms seen in the field or on tubers is understood before a management strategy is put in place.

Testing for the presence of FLN and TRV

Soil can be sampled for the presence and number of the main plant parasitic FLN species present. The researchers involved in a TSB/AHDB- funded project[1] have made the following recommendation regarding sampling:

1. If the 10 cm soil temperature has been at 0^oC or below for five consecutive nights, do not sample.
2. If there has been a prolonged spell of warm/hot weather, resulting in soil temperatures around 20^oC, coupled with low rainfall for 3-5 days, do not sample.
3. The minimum recommended sampling rate is one composite sample ha^{-1}
4. Each composite sample should be comprised of at least 50, ideally 70 small cores. These figures are minimum values. A soil sample weighing at least 500g should be generated.
5. The area to be sampled should be walked in a W shape with sampling points randomly located along the W.
6. At each sampling point, detritus on the soil surface such as dead plant material should be removed prior to sampling.
7. A trowel or sampling implement that can sample to a depth of 15 cm is acceptable (for trichodorid nematodes, soil to a depth of 15 cm should be taken). The implement should have at least a 25mm (1 inch) diameter. Do not use an auger as a screwing action can damage FLN.
8. Soil should be placed in labelled plastic bags (ideally zip-lock bags) and moved to storage as soon as practicable. Include a clearly written label (using an indelible pen) along with the soil as a backup label.
9. Samples should be stored in a cold store at a temperature of 4^oC. Do not store samples in a fridge that has a freeze-thaw cycle. (Many modern fridges have this cycle as default).
10. For mailing, place the samples in a strong cardboard box with some loose packing material.

Laboratories offering commercial soil testing also provide their own guidance on the number of samples to be taken. There is a need to be aware of differences in soil type and sub-divide fields into units to take account of these. FLN will move vertically in the soil profile mostly following the water table and as such populations can appear to be highly variable over time. Soil sampling is usually carried out between October and March. It is recommended not to sample in waterlogged conditions; soil should preferably be moist but not wet at the time of sampling.

Some laboratories can also provide information on whether TRV is present within the sample of FLN obtained during soil sampling. 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

[1] "Strategies for Quantifying and Controlling Free Living Nematode Populations and Consequent Damage by Tobacco Rattle Virus to Improve Potato Yield and Quality".

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). Of the two approaches, the bait plant technique will take longer to complete.

FACTORS TO BE AWARE OF WHEN CONSIDERING THE POTENTIAL FOR DIRECT FEEDING DAMAGE AND/OR TRV SPRAING SYMPTOMS:

FIELD SELECTION

Is there a history of TRV spraing or confirmed FLN feeding damage?

The lack of previous history of TRV spraing is not always a good indicator of the risk of the problem occurring. Whether TRV symptoms are seen will be influenced by the variety grown and whether a nematicide was used. However, once a field is infected with TRV the virus will persist in weed hosts and volunteers and will be very difficult to eliminate. Delayed or non-emergence in previous potato crops may indicate a potential FLN direct feeding damage problem but could also be due to other causes such as infection by *Rhizoctonia solani*. There is no currently known varietal resistance in potatoes to direct feeding damage caused by FLN.

What soil types are present in the field?

The activity and movement of FLN are affected by soil structure, aeration and moisture. In general, sandy soils including sandy clay loams, are considered to be soils where greater problems with nematode feeding damage and TRV spraing occur, compared with loam soils and silts and soils with a high clay content.

Compaction will restrict root growth and is likely to exacerbate the adverse effects of FLN direct feeding damage.

When will the crop be planted?

Early planting (March/early April) when soil temperatures are low (e.g. below ~8^{o}C) will lead to prolonged emergence and the possibility of greater direct feeding damage compared to later planted crops (late April/May) where soil temperature is higher and generally rising; emergence will be more rapid and the plants' ability to compensate for the effects of feeding damage will be greater.

Is there evidence of the presence of potato pathogens in the field?

There is information from both GB and overseas that interactions between nematodes and plant pathogens such as *Verticillium*, *Fusarium* and *Rhizoctonia* can have a significant impact on potato growth. In the US, potato early dying syndrome is associated with *Verticillium dahlia*. The fungus and *Pratylenchus penetrans* can act in combination causing severe symptoms at population levels that have little or no effect when each species is present individually.

Are FLN/TRV soil test results available?

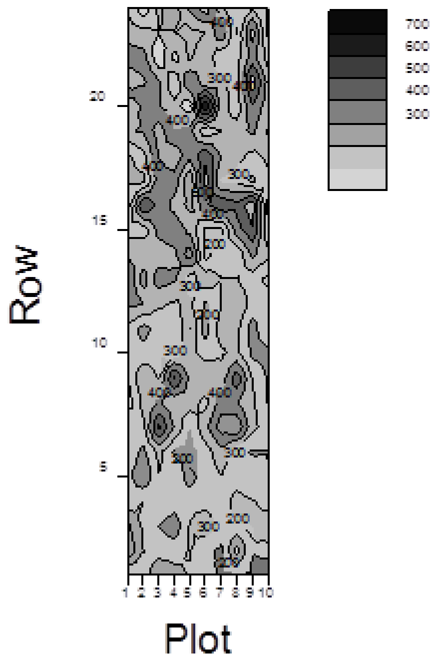
As mentioned above, several laboratories in the UK offer FLN and TRV diagnostics services. There is limited published information on the relationship between the numbers of FLN present and direct feeding damage. As explained below, the results from the trials completed in work package 2 (of the TSB/AHDB-funded research project on FLN) do not support the establishment of simple damage thresholds for direct feeding damage by FLN.

EXAMPLES OF VARYING RISKS OF DIRECT FEEDING DAMAGE BASED ON FIELD TRIALS

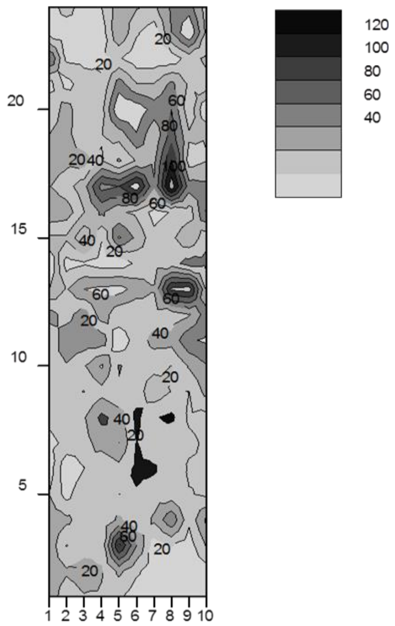
Within the research project, the performance of 12 potato varieties was studied in replicated plot trials at four sites (Perthshire, Yorkshire, Norfolk, Shropshire) in each of three years. The FLN counts reported from individual plots ranged from 1 to 870 trichodorids and 0 to 141 *Pratylenchus* species per 200 g soil. Replicate plots were either treated with Vydate 10G (55kg/ha, incorporated) or received no nematicide. In each site/year the trial design required that all varieties were harvested at the same time. In these circumstances it has not been possible to identify a consistent effect of FLN numbers on marketable yield.

Examples of results from different sites in the same year are provided below. The contour maps show how the numbers of trichodorids (*Pratylenchus* numbers are not included in the figures) varied across the experimental area at each site. Each individual replicate was 6.25m long by 4 rows wide, with a 1.5m discard between plots along the row.

York Nematode Counts



Norfolk Nematode Counts



The average FLN counts and range of nematode counts (in 200g of soil) from the 240 trial plots sampled prior to planting at the two sites in 2011 were:

	Yorkshire		Norfolk	
	Trichodorids	*Pratylenchus* species	Trichodorids	*Pratylenchus* species
Mean ± SE	321.0 ± 8.7	5.4 ± 0.4	36.4 ± 1.9	3.0 ± 1.1
Range	103 - 870	0 -38	2 -163	0 - 36
Planting date	7th April 2011		22nd March 2011	

There were no differences in emergence between any treatments at the Norfolk trial. In Norfolk, there was a difference in ground cover between Vydate-treated and Untreated plots, but only for one variety (Markies).

In Yorkshire, untreated Pentland Dell, Saxon and Maris Piper took significantly longer to reach 50% emergence compared to the same varieties in plots treated with Vydate 10G. The number of days from planting to 50% emergence was in the range 29 to 36 days.

In Yorkshire, 40% of varieties had a significantly greater ground cover in plots treated with Vydate. These were Pentland Dell, Shepody, Linton, Saxon, Melody, Sylvana and Harmony. However, when marketable yield (>45mm) was analysed differences (in terms of higher yields) between Vydate-treated and untreated plots were only seen in Saturna (Norfolk) and Melody (Yorkshire).

Given that the Yorkshire site had high trichodorid numbers, an analysis of the interaction between the mean yield and the nematode count in each individual plot was carried out. There was no consistent relationship with treatment or trichodorid counts for any variety in terms of tuber yield.

The factors at each site are summarised in the table below. This is intended to show that depending on the how the various factors and growing conditions combine the risk of losses in marketable yield (**due to direct feeding damage**) can vary. The tables refer to low, medium or high populations of trichodorids. These are arbitrary categories based on the range of average trichodorid numbers recorded during the project.

Low: average of 1-75 trichodorids per 200g soil
Medium: 76-150 trichodorids per 200g soil
High: more than 150 trichodorids per 200g soil

In themselves they do not indicate the risk of damage occurring but are simply used to provide a broad description of the number of trichodorids at each site. Other factors need to be taken into account to assess the risk of damage occurring.

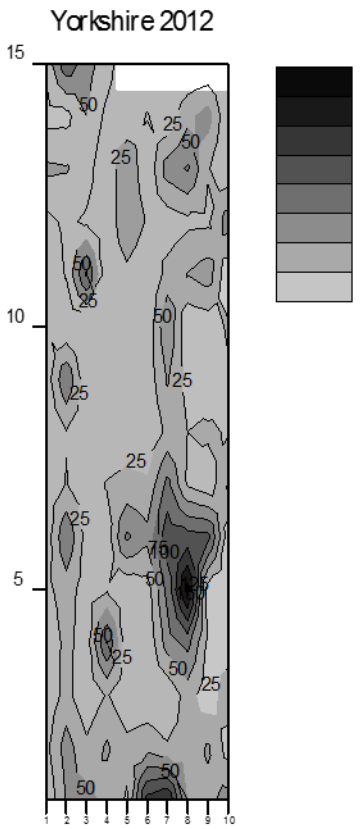
In most cases we recorded less than 10 *Pratylenchus* species per 200g soil and the sites are described as having a low population of *Pratylenchus* species.

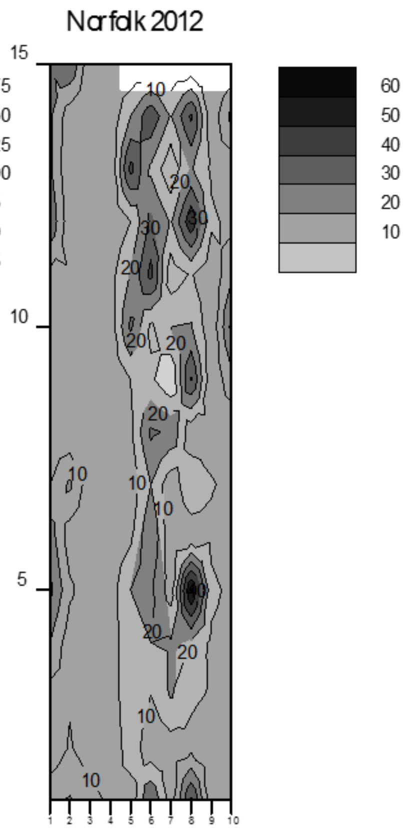
Yorkshire	Norfolk
High population of trichodorids	Moderate population of trichodorids
Low population of *Pratylenchus* species	
‘Average’ planting date	‘Early’ planting date
Emergence 29 to 35 days	Slower emergence (34 to 36 days)
Nematicide application in treated plots: Vydate 10G 55kg/ha	Nematicide application in treated plots: Vydate 10G 55kg/ha
Taking into account the information available, the risk of direct feeding damage was considered to be:	
Medium/High Risk (based on the average planting date and high trichodorid numbers)	Medium Risk (based on the early planting date and moderate trichodorid numbers)

Outcomes:	
Nematicide increased ground cover significantly in a range of varieties (Pentland Dell, Shepody, Linton, Saxon, Sylvanna and Harmony). These are generally determinant varieties.	Nematicide increased ground cover significantly in just one variety (Markies).
Effects on yield (total and marketable) were generally insignificant with only one variety at each site where yield was improved by the application of a nematicide.	
Comments:	
High numbers of trichodorids with effects noted on some varieties early in the season. However, effects on later growth and yield were minimal.	Lower average numbers of trichodorids which might have affected crop growth if conditions had been poor following the relatively early planting date. However, in this trial no significant effects on marketable yield from application of the nematicide were observed.

More details of the trials are available in the project final report (see Work package 2 Final Report).

A similar approach (with fewer varieties) was repeated in 2012. Contour plots showing the distribution of trichodorid counts from two of the trial sites are shown below.





The average FLN counts and range of nematode counts (in 200g of soil) from the 144 trial plots sampled prior to planting at the two sites in 2012 were:

	Yorkshire		Norfolk	
	Trichodorids	*Pratylenchus* species	Trichodorids	*Pratylenchus* species
Mean ± SE	32.9 ± 2.7	0.56 ± 0.13	12.2 ± 0.1	0.42 ± 0.2
Range	1 - 185	0 -8	1-62	0 - 12
Planting date	16th May 2012		28th March 2012	

In Yorkshire in 2012, the first assessment (12th June) was completed after 50% emergence in most varieties. In Norfolk, it was untreated Innovator that showed a delay in time to 50% emergence compared to the same variety treated with Vydate 10G. At this site, the emergence was slow taking from 43 to 51 days to achieve 50% emergence. However, there were no significant differences in marketable yield (> 45 mm) between the Vydate 10G-and untreated treatments for any variety at either the Yorkshire or Norfolk trial site.

Yorkshire	Norfolk
Low population of trichodorids	
Low population of *Pratylenchus* species	
'Late' planting date	'Early' planting date
Rapid emergence (less than 27 days)	Prolonged emergence (43-51 days)
Nematicide application in treated plots: Vydate 10G 55kg/ha	Nematicide application in treated plots: Vydate 10G 55kg/ha
Taking into account the information available, the risk of direct feeding damage was considered to be:	
Very Low Risk (based on the late planting and low trichodorid numbers)	Low Risk (based on the early planting and low trichodorid numbers)
Outcomes:	
Only one variety (Harmony) exhibited a significant difference in the percentage ground cover between the Vydate-treated and untreated plots.	Seven varieties (M. Piper, P. Dell, Shepody, Markies, Innovator, L. Rosetta, Crisps4All, Harmony) had a greater percentage ground cover in the Vydate-treated compared to the untreated plots
Effects on yield (total and marketable) were insignificant across all varieties	

Comments:	
Low average numbers of nematodes present with no effect on crop growth or yield.	Low numbers of nematodes present which even in a situation where emergence was prolonged did not ultimately affect yield.

Although these results are a subset of all those collected in the research project they provide an indication of the difficulty in determining the risk posed by FLN feeding damage in different situations.

Overall, when the risk of FLN direct feeding damage is being considered, it is proposed that FLN counts are only one component of the potential risk and all the other crop and site related risk factors should be taken into account.

RISK OF TRV SPRAING DEVELOPING

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 trichodorid nematodes are detected in a soil sample it is recommended that tests are carried out to determine if the nematodes are carrying TRV. The results of these tests should be considered in conjunction with the available information on the variety that is intended to be planted at the site.

VARIETY

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

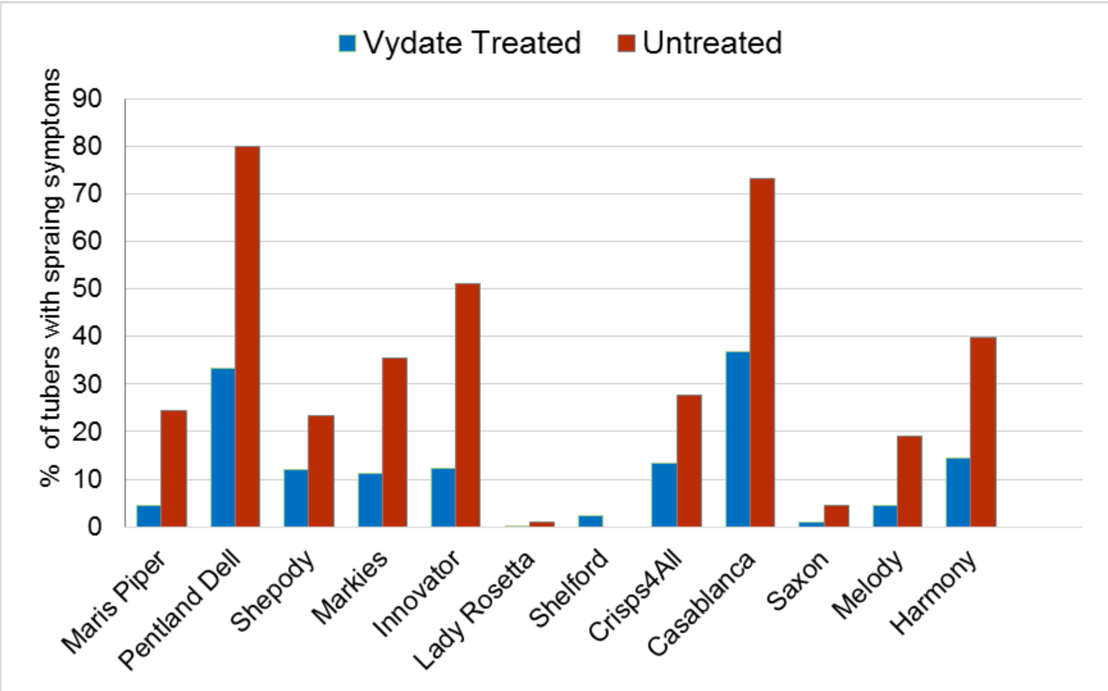
- **Resistant**: the varieties do not show any symptoms and virus particles cannot be detected in the plant, including in the tubers.
- **Spraing sensitive:** varieties which exhibit spraing symptoms in the tubers and/or external necrotic lesions and malformations. Virus particles are rarely found in 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). Infected tubers can act as a reservoir of TRV and result in the movement of the virus to new sites at 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. The ratings do not differentiate between resistant varieties and TRV susceptible varieties as the latter may not show symptoms in the tuber flesh. The testing has not been part of the programme of National List tests for many years so that for newer varieties there are no official ratings for TRV spraing resistance reported on the Potato Variety Database or in the NIAB TAG pocket guide to varieties. In this case ask your agronomist or breeders for information.

Data on TRV spraing is available from the field trials discussed above. In each case the numbers of tubers with symptoms of spraing out of 50 tubers per plot for each variety at the sites, was recorded. Tubers were tested for the presence of TRV and Potato Mop-Top Virus (PMTV).

FIELD TRIALS

TRV spraing was not found at either the Norfolk or the Yorkshire trial sites in 2011. TRV spraing was recorded at the Yorkshire site in 2012 (it was a different field to that used in 2011). The results shown refer to the presence or absence of necrotic arcs in the tuber flesh. Other potential impacts of FLN damage, such as 'spots and marks', are not included in the figures. In the trials, plots of each variety were left untreated or treated with Vydate 10G (55 kg/ha).



Mean percentage of tubers exhibiting spraing (TRV) symptoms at harvest at the Yorkshire site (2012).

Maris Piper, Pentland Dell, Innovator, Casablanca and Melody all had significantly lower levels of TRV spraing in the Vydate 10G treatment.

There was a third location (in Shropshire) where different parts of the same field were used for variety trials over a three year period. 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. Significant levels of spraing were recorded in all three years.

The average FLN counts and range of nematode counts (in 200g of soil) from the trial plots sampled prior to planting at the Shropshire site in 2011, 2012, 2013 were:

Shropshire		
Soil type: Light sand		
2011	Trichodorids	*Pratylenchus* species
Mean ± SE	43.8 ± 1.9	7.4 ± 0.6
Range	2 - 129	0 - 57

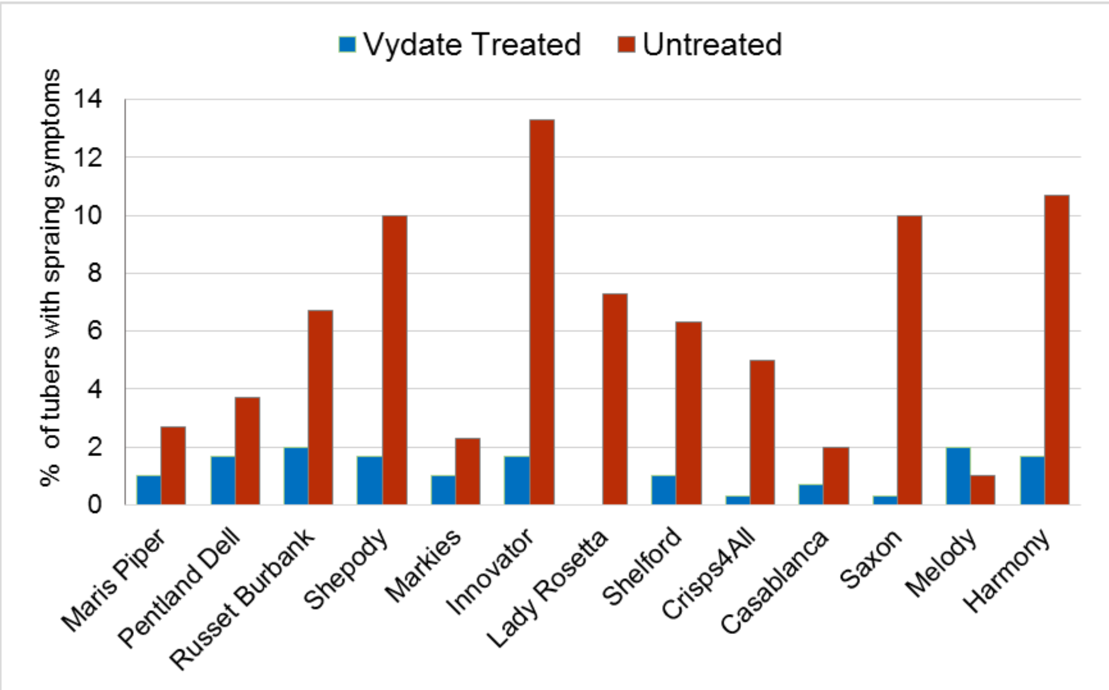
2012	Trichodorids	*Pratylenchus* species
Mean ± SE	8.3 ± 0.6	34.8 ± 2.3
Range	0 -37	2 -141
2013	Trichodorids	*Pratylenchus* species
Mean ± SE	19.6 ± 1.6	40.2 ± 2.9
Range	0 - 84	2 - 141

(*Pratylenchus* species are not vectors of TRV but the numbers recorded in each year have been provided for general information)

2011: TRV spraing

The percentage of tubers showing TRV spraing varied between varieties. In all varieties (except Melody) the application of a nematicide (Vydate 10G 55kg/ha) reduced the incidence of TRV spraing. The reduction was statistically significant in all the varieties except Maris Piper, Pentland Dell, Hermes, and Casablanca.

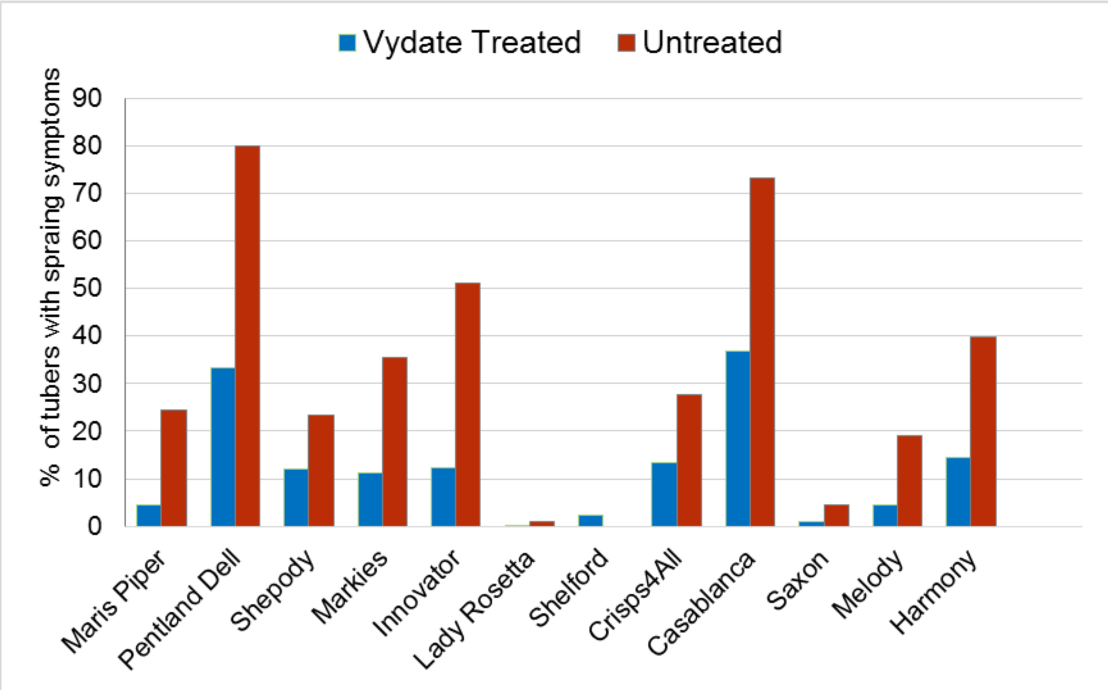
Analysis of the number of potato tubers exhibiting spraing symptoms in each plot against the trichodorid count did not show any correlation between the nematode population and the likelihood of spraing symptoms (i.e., a high incidence of spraing symptoms can occur in plots with low trichodorid numbers).



Mean percentage of tubers exhibiting spraing (TRV) symptoms at the Shropshire site in 2011.

2012: TRV spraing

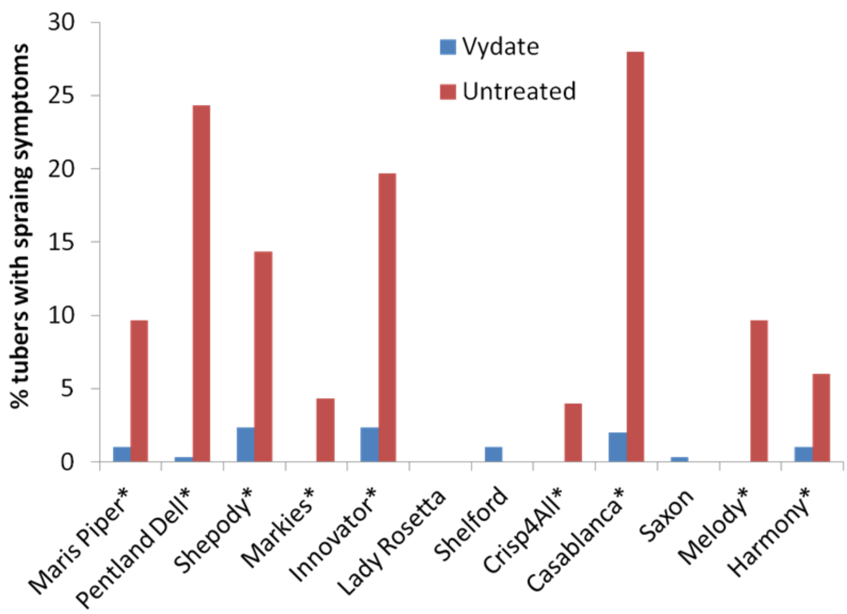
All varieties except Lady Rosetta and Shelford had significantly lower levels of spraing in the Vydate 10G treatment (P < 0.05). The results reinforce the comment that low trichodorid numbers can still represent a high TRV spraing risk, as the average trichodorid count in the trial plots was 8 per 200g soil.



Mean percentage of tubers exhibiting spraing (TRV) symptoms at the Shropshire site in 2012.

2013: TRV spraing

In 2013, all varieties except Lady Rosetta, Shelford and Saxon had significantly lower levels of TRV spraing in the Vydate 10G treatment (P < 0.05).



Mean percentage of tubers exhibiting spraing (TRV) symptoms at the Shropshire site in 2013.

SUMMARY

The factors to consider when assessing the risk posed to potatoes by FLN are provided below. Items in red are considered high risk situations where action is required. This could be nematicide use, changing the variety to be grown in the field or not using the field for potato production. Some, or all, of the factors in orange may also combine to generate a high risk situation requiring action.

(i) Direct feeding damage

Previous history of confirmed FLN feeding damage.

Sandy soil, or heavier soils but compaction present.

FLN test results available and the test provider has indicated that FLN are present and may pose a risk. (Ensure the soil sample was taken under appropriate conditions and is representative of the whole field).

Early planting (March/early April) of the crop.

Other problems known to be present (*Rhizoctonia, Verticillium*) in the field.

(ii) Transmission of TRV and TRV spraing symptoms develop

Previous history of confirmed TRV spraing

FLN/TRV test indicates presence of the virus and a spraing sensitive variety is to be grown

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[1] TSB/AHDB-funded project: the results discussed in this summary were obtained during a five year research project “Strategies for Quantifying and Controlling Free Living Nematode Populations and Consequent Damage by Tobacco Rattle Virus to Improve Potato Yield and Quality”. The project was was co-funded by Technology Strategy Board and AHDB Potatoes. The project partners were Cygnet Potato Breeders Ltd, McCain Foods Ltd, PepsiCo International, DuPont, The Cooperative Farms, Eden Research, Mylnefield Research Services Ltd, James Hutton Institute, SAC, Plant Health Care UK Ltd and Tozer Seeds Ltd in conjunction with Harper Adams University.