Research Review

Free Living Nematodes and Spraing

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Summary for levy payers

Tobacco rattle virus (TRV) causes a number of different symptoms in potato plants including necrotic arcing (known as spraing, corky ringspot) in the tuber flesh, and stem-mottle (distortion, stunting and mottling) and aucuba in the foliage. The virus is transmitted by trichodorid nematodes, and has a wide host range, including many common agricultural weed species. These weeds serve to maintain the virus in a field and its nematode population, in a perpetual cycle of transmission and acquisition. The distribution of the virus within a field is often patchy, and reflects that of its nematode vectors, which prefer light and/or sandy soils.

This desk study aimed to assess the information on the principal Free Living Nematodes (FLN) in the UK, including their biology, distribution, economic importance and management. Principal nematodes within the project were the main vectors of TRV within the UK, namely: *Trichodorus primitivus, Paratrichodorus pachydermus* and to a lesser extent *P. anemones,* the viral cause of 'spraing' or corky ring spot. Other free living nematodes constituted an appropriate minor part of the project and included *Longidorus elongatus*, *Longidorus macrosoma* and *Pratylenchus penetrans.*

Information on the distribution of viruliferous nematode populations within the UK is extremely patchy and disjointed. This study has gathered information collected over the past 10 years by the principal researchers and advisors working in the industry, including CSL at York, SAC based in Edinburgh and SCRI at Dundee, with further information from limited questionnaire returns from growers and industry representatives and also limited information from the chemical/nematicide industry.

Different estimates are available regarding the proportions of Trichodorid and Paratrichodorid nematode populations that appear viruliferous. Parker (ADAS, 2005) estimated that in Scotland, the nematodes were in 65% of lighter, or sandier, soils and were in up to c.33% of lighter soils in England, of which 80% were believed to be carrying the virus.

Evans (2006) estimated that 30% of fields were viruliferous, based on 311 samples. Evans estimated 30 to 35% for soil samples from Lothians, Borders and Fife, with lower proportions from Perthshire and elsewhere, though sampling will have been affected by advisor/grower knowledge targeting known suspect areas in both cases.

CSL identified and confirmed the distribution of the virus in the lighter sandier soil within the principal potato growing areas of Yorkshire (mainly north), East Anglia (Norfolk, Lincolnshire and Cambridgeshire), West Midlands (mainly Shropshire and a few other confirmed sites in East Midlands (Notts, Leicestershire), Isle of Wight, Isle of Man and south west of England.

In Northern Ireland Tobacco Rattle Virus has been relatively uncommon or unreported, but has been detected in Magilligan (Co Londonderry), Maze and Drumbeg (Co. Antrim), and Sandhill (Co. Tyrone). In the last few years spraing caused by TRV has also been found in several parts of Co. Down.

The distribution of the virus was confirmed to the report authors through the data collected from the various agencies and individuals, with the principal 'hotspots' focused

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largely as expected in areas of lighter soils including, in Scotland, the areas surrounding Elgin, the Angus area around Forfar – Brechin in the Vale of Strathmore, parts of Perthshire, through Fife and into the East Lothian area. SCRI records (Neilson, 2006 pers comm) indicated that out of an area from Northumberland northwards, largely on the east coast of Scotland, some 31% of samples were positive for TRV. Estimates using the 41% of grower returns which detailed the area affected indicated that 560.6 ha out of 1288.6 hectares had tested positive for the virus.

From the collected reports and limited data available from the various agencies and chemical companies, an estimate of c. 20,000 to 24,000 ha., or c. 12% of the potato area appears to be infected to an extent. Distribution maps of the principal nematodes and other notable FLN in the UK are presented, as is a distribution map of reports of TRV within the UK.

Full details of the biology, life cycles, distributions and known crop damage/effects are presented. Details of direct economic losses due to nematicide application and crop losses alone give a conservative estimate of £2.2 million per annum to the potato industry.

Options for the control of FLN and also of TRV are limited and are discussed, with nematicides serving as the principal control at present. With the withdrawal of aldicarb in the near future, increasing controls on use of alternative chemical control methods and pressure from consumers /supermarkets to justify and reduce chemical inputs it is evident that the industry needs to examine alternative measures. The situation is further exacerbated by weed growth in set-aside land and by weeds within organic farming systems.

The report reviews the use of nematicidal plants to reduce FLN. There is little research in the UK in this area, but an increasing amount of research in the US, though environmental conditions are somewhat different, the approaches merit investigation within a UK context.

Existing methods of determining the occurrence of TRV rely either on counts of trichodorid numbers, which do not necessarily correlate with the presence of virus, or on detecting the virus in bait plants grown in samples of soil. Traditional bait tests are reasonably reliable, but take more than a month to complete, require large amounts of glasshouse space and can be a significant cost to growers. Therefore, the number of samples taken per field is usually quite small, leading to poor definition of affected areas and the possibility of missing the virus altogether. A development of the bait test, now being offered through the Central Science Laboratory at York, uses real time quantitative polymerase chain reaction (TaqMan) technology to detect the virus in the roots of the bait plants. Another new diagnostic, developed at SCRI and available through SAC, uses improved nematode extraction systems and TaqMan technology to detect viruliferous nematodes. BPC funded work (R255 –"TRV - improvement of a diagnostic test to allow more precise localisation of Tobacco Rattle Virus in fields") has identified the potential of using a number of common weeds as indicators of the presence of the virus. The results are promising in regard to identifying highly viruliferous areas – or 'hotspots' – that appear to agree with spraing symptoms in the following potato crop. However, the areas of lower population levels, or lower viruliferous numbers of nematodes, and the subsequent symptom expression are difficult to predict with accuracy and probably rely on a number of environmental factors. This problem of correlating diagnostics to subsequent symptom expression in following potato crops applies to all

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current diagnostic procedures and is exacerbated by the fact that c.1% to 2% infection in seed crops or 4% to 6% in some ware/processing crops may cause rejection of the crop. As such the usefulness of the weed test and the other tests available and their predictive application needs further field trialing to allow growers a degree of confidence and allow decision making with regard to management options.

Resistance to Tobacco Rattle Virus within cultivars suitable for the many diverse and specialised markets is a sustainable option for the long-term objectives for the industry. While such resistance has been identified, further work is needed to bring it to the market place in suitable varieties.

Practical recommendations for growers.

The report maps the distributions of the free living nematodes and also of Tobacco Rattle Virus within the UK. It is unlikely that further significant spread of these nematodes is occurring. Movement of soil on machinery and through waste soil is still a possibility. The epidemiology of Tobacco Rattle Virus is now resolved, with the virus being entrenched within many weed species in arable fields and thus acting as a virus reservoir by which feeding nematodes become viruliferius and transfer the virus to a potato crop.

The choice of variety is often limited by the grower’s commitment or requirement to meet buyer requirements. However, the choice of resistant varieties (8 or 9 on NIAB scale) where available would ease requirements for nematicide use. Some growers still need to be fully informed that there is a risk that certain varieties (see Table 2) can become systemically infected and transferring the virus between fields on farm and between farms and that the virus can be transferred systemically over many years.

Full details with regard to the effect of TRV in the potato crop and best practice for growers are given in advisory notes in Appendix 1.

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Introduction

Plant parasitic nematodes are microscopic in size, many barely if at all visible to the naked eye, which explains why they are often overlooked as causal agents of poor plant health. In addition, the above ground symptoms are typical of a large number of infectious and non-infectious root diseases.

Direct feeding by nematodes can drastically decrease a plant's uptake of nutrients and water. Nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination e.g. in carrots and parsnips (Ploeg, 2001). Nematode feeding also creates open wounds that provide entry to a wide variety of plant-pathogenic fungi and bacteria. These microbial infections can often be more economically damaging than the direct effects of nematode feeding.

There are many migratory nematode pests of arable crops, including potatoes, which commonly cause plant injury including *Heterodera* spp., *Globodera* spp., *Xiphenema* spp., *Trichodorus* spp., *Paratrichodorus* spp., *Longidorus* spp., and *Paratylenchus* spp.. Figure 1 below illustrates the relative sizes of the different nematodes.

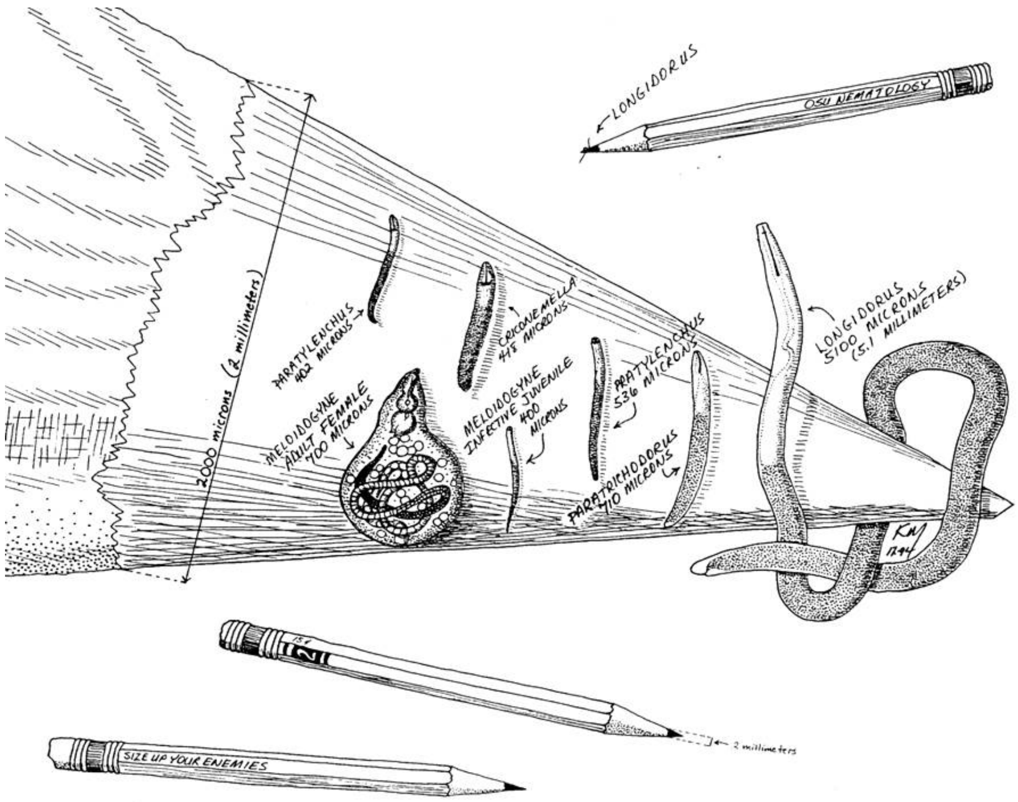

FIGURE 1. MODIFIED FROM RUSSELL INGHAM AND KATHY MERRIFIELD 1996.
A Guide to Nematode Biology and Management in Mint. *Integrated Plant Protection Center, Oregon State University, Corvallis. Pub. No. 996. 38 p*

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Except for *Heterodera* spp., most are of the migratory type. That is, they are mobile in the soil and move from feeding site to feeding site on the host plant and also between the roots of different host plants. When crops show slow decline, lack of vigour, chlorosis or slower than normal growth, nematodes may be the cause. During periods of stress (or even hot spells) or nutrient deficiencies, infested plants will tend to be affected first. Other symptoms include a crooked or brushy appearance of fleshy tap roots, stunted, stubby, small root systems with excessive branching; small roots larger near the tip; sparse lateral roots; brownish to black spots or streaks or discoloured necrotic areas on the roots. The damage that such nematodes can cause varies with species and generally increases with the numbers of nematodes present. However, the economic damage caused by some free living nematodes through feeding on roots may be exacerbated by other organisms invading or transferring through the feeding sites (e.g. bacterial, fungal or, **notably,** viral). Plants attacked by nematodes are also often more susceptible to other unfavourable environmental conditions such as drought. The yield losses through direct feeding of free living nematodes on potato roots is unknown and difficult to estimate as are the associated costs to the UK potato industry. Estimates of the relationship between detectable crop damage and population size thresholds are presented below in Figure 2 (Bill Parker, ADAS 2005). It is evident that the longidorid nematodes reach the damage threshold at lower population levels, followed by the trichodorid nematodes, though crop loss may be significantly higher when the latter acts as vector of Tobacco rattle virus, in which case notably lower population levels may cause severe crop losses due to corky ring spot / spraing symptoms.

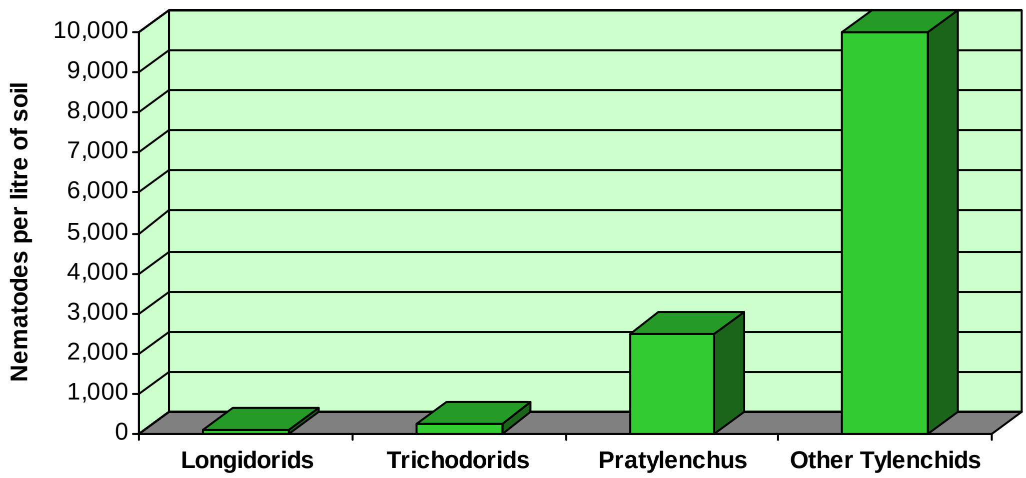

FIGURE 2. POPULATION LEVELS FOR DETECTABLE DIRECT CROP DAMAGE (PARKER, ADAS)

Spraing symptoms may also be caused by infection with Potato Mop Top Virus *(see photograph below)*, which is transmitted by the fungal pathogen *Spongospora subterranea* (causal pathogen of powdery scab) and is not included in the current review.

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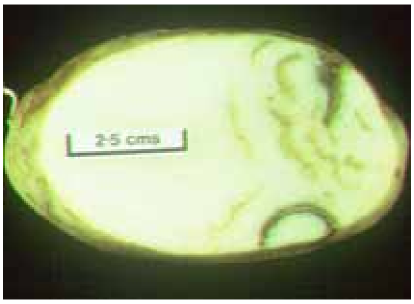
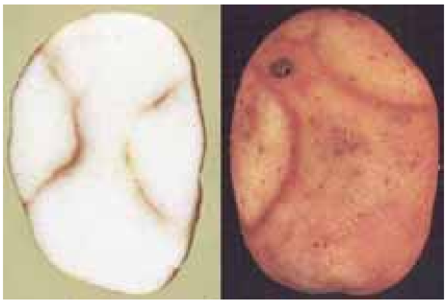


Spraing symptoms in tuber due to TRV



Spraing symptoms in tuber due to Potato Mop Top Virus

Light, sandy soils generally favour larger populations of plant-parasitic nematodes than clay soils. Free living nematodes live on the thin film of water that surrounds each soil particle and are, thus, very sensitive to dry soil conditions. Nematodes move very slowly in the soil but are moved in running water or on soil contaminated equipment (Boag, 1985).

Numbers of the potential spraing-causing Trichodorid nematode in soil have increased by up to x3 over the past decade, according to the Scottish Agricultural College (Evans, 2006). The population levels of the different nematodes fluctuate significantly between years as seen in the figures from SAC surveys (Evans, 2006) in Table 1 below.

TABLE 1 PERCENTAGE OF NEMATODE POPULATIONS WITHIN 3 POPULATION CATEGORIES.

	% of Nematode counts within each population category*								
	Longidorids			Trichodorids			Pratylenchids		
Year	0-24	**25-99** [a]	**>100**	0-99	**100-199**	**>200**	0-99	**100-199**	**>200**
94-95	47	**37**	**16**	79	**10**	**11**	77	**8**	**15**
95-96	56	**28**	**16**	71	**16**	**13**	26	**21**	**53**
96-97	51	**37**	**12**	59	**20**	**21**	29	**14**	**57**
97-98	54	**32**	**14**	58	**17**	**25**	29	**29**	**42**
98-99	72	**22**	**6**	48	**23**	**29**	14	**34**	**52**
99-00	62	**27**	**11**	49	**19**	**32**	23	**45**	**32**
00-01	64	**28**	**8**	62	**21**	**17**	31	**17**	**52**
01-02	63	**26**	**11**	61	**19**	**20**	24	**27**	**49**
02-03	65	**26**	**9**	56	**18**	**26**	33	**21**	**46**
03-04	58	**31**	**11**	61	**17**	**22**	18	**25**	**57**
04-05	69	**24**	**7**	49	**19**	**32**	16	**21**	**63**

* Population categories - e.g. 0-24 in 250 g/soil.
[a] Categories in bold signify percentage of samples with potentially damaging populations of nematodes.
From: K.A.Evans, 2006.

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These observations of fluctuations in the different nematode population levels across the years in the above table suggest that c. 50% of the populations could be sufficiently high to result in direct feeding damage. The problem of direct root damage will not be isolated to potatoes, as feeding damage has been witnessed in other crops, including cereals and carrot crops.

Apart from the direct feeding/ physical damage to the roots of plants including potatoes, the Trichodorid free living nematodes are the vectors of Tobacco rattle virus (TRV), a *Tobravirus* which causes corky ringspot or 'spraing' disease. The nematodes occur more often in coarse sandy soils. Tobacco Rattle Virus (TRV) can cause a number of different symptoms in potato plants including necrotic arcing (known as spraing, corky ringspot) in the tuber flesh, and stem-mottle (distortion, stunting and mottling) and aucuba in the foliage. Symptoms in **virus susceptible** varieties appear on the foliage, the appearance may be transient, with some symptoms in the tuber. However the tubers of **spraing susceptible** varieties contain corky layers of tissue interspersed with rings of healthy tissue and brown flecks distributed throughout the tuber.

TRV has a wide host range, particularly among weed species, potentially infecting more than 400 monocotyledonous and dicotyledonous species across over 50 families. The virus does not usually become systemic in most of these hosts, often remaining in the roots. However, several species can become systemically infected and some, such as *Stellaria media*, may show no obvious foliar symptoms. The virus host range includes many common agricultural weed species (Cooper *et al*, 1971), and these weeds serve to perpetuate the virus within sites and the nematode populations such that when the virus become established within the field weed population, it is virtually impossible to eradicate and 'clean up' the local weed population. Distribution of the virus in the soil reflects that of its free living nematode vectors, which prefer light and/or sandy soils.

Potato cultivars differ in their propensity to develop spraing symptoms when exposed to viruliferous nematodes; some, such as Pentland Dell and Maris Bard, are highly susceptible and exhibit classic spraing symptoms as illustrated above, whereas others, such as Record, are resistant to infection by TRV. However, Xenophontos *et al.* (1998) and Dale *et al.* (2000) demonstrated that some cultivars that rarely exhibit spraing symptoms, such as Wilja, can become infected with TRV without developing spraing symptoms. This type of infection differs in several ways from that in spraing-susceptible cultivars. Thus, infections associated with spraing symptoms tend not to become fully systemic and are transmitted to only a proportion of daughter tubers. In contrast, TRV infections in cultivars that exhibit few, if any, spraing symptoms tend to be transmitted to all daughter tubers and can persist through many vegetative generations with virus recoverable from all parts of the plants. Moreover, such plants can serve as sources for virus acquisition by vector nematodes (Xenophontos *et al.* 1998) and hence serve to distribute the virus over considerable distances to new sites, if the appropriate nematodes are present (Dale *et al.*, 2000). Transfer of the virus through seed transmission within certain potato varieties to new fields/sites has probably occurred over the past century of movement of seed and reflects the current quite extensive distribution of the virus within the UK and European arable land.

Few satisfactory methods are available for the control of TRV infection, and the situation will become worse with the imminent withdrawal of aldicarb, one of the principle chemicals used to control the activity of the trichodorid nematodes that transmit the virus.

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This project aimed to assess the information on the principal free living nematodes in the UK currently available, including their biology, distribution, economic importance and management. Principal nematodes within the project will focus on the main vectors of TRV within the UK, namely: *Trichodorus primitivus, Paratrichodorus pachydermus* and to a lesser extent *P. anemones.* Other free living nematodes will constitute an appropriate minor part of the project and will include *Longidorus elongatus*, *L. macrosoma* and *Pratylenchus penetrans.*

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Free Living Nematodes – Trichodorids

Species and biology

Free-living nematodes (FLN) are those nematodes which do not have a phase of their life cycle within a host and represent the majority of known nematode species/genera (Hugot *et al*., 2001). Frequently non-specialists refer to FLN as meaning plant-parasitic nematodes that are not *Globodera pallida* or *G. rostochiensis*, however, FLN is a global term with species/genera that represent each of the nine recognised trophic groups (Yeates *et al.*, 1993), e.g. bacterivores, fungivores and predators.

Globally nematode damage has been conservatively estimated to cost the agricultural sector > $100 billion per annum (extrapolated from Sasser and Freckman, 1977) and in economic terms seven of the ten most economically damaging nematode genera are free-living including *Paratrichodorus* and *Xiphinema* both of which have known virus-vector species.

The longevity of FLN within a field context, application of nematicides notwithstanding, is dependent upon soil type, presence of a suitable host, temperature and soil moisture (Taylor *et al.*, 1978; Brown and Coiro, 1983). For virus-vectors species including those which vector TRV, soil temperature is considered to be the main factor limiting geographic distribution (Boag *et al.*, 1991). Within the UK, optimum environmental conditions for trichodorid species including most of the virus-vector species is a sandy loam or loamy sand soil with a sand fraction between 80-90%, a silt fraction of < 10% and with an altitude of < 70m (Alphey and Boag, 1976).

***1. Trichodorus primitivus* (de Man, 1876) Micol. 1922 – Type species**

Family:	Trichodoridae
Common name:	stubby root nematode
Vector of viral disease:	corky ring spot of potatoes, spraing

Hosts

The nematode has an extremely wide host range including alfalfa, azalea, boysenberry, vegetables, corn, tomato, onion, wheat, sugarcane, rice, grasses, and many weeds. Nematodes are vectors of Tobacco Rattle Virus. Poor hosts include orchard grass, rye, spinach, radish, strawberry, pea and tobacco. Non-hosts are asparagus, showy crotalaria, poinsettia and jimsonweed.

Life Cycle

T. primitivus is a migratory ectoparasite. The nematodes can complete their life cycle in 16-17 days at 30°C (86°F), 21-22 days at 22°C (72°F). Temperature range for reproduction is 15 to 30°C (68-95°F). Males are rare. In favourable conditions nematodes can have extremely high multiplication rates. They feed over the whole of the root surface, but usually close to the root tip, including the root cap, the meristematic region, and the region of elongation; the nematode pierces the epidermal cell walls and root hairs with rapid thrusts (10 per second).

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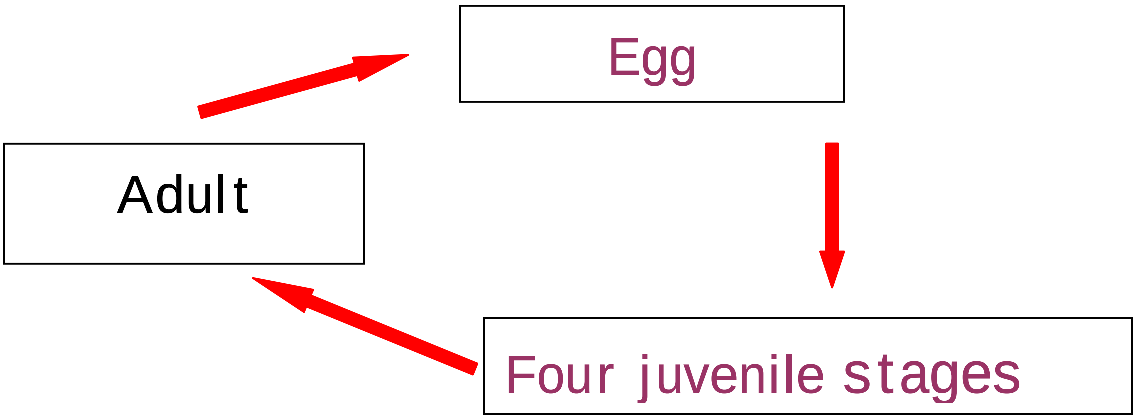

FIGURE 3A SCHEMATIC LIFE CYCLE OF *T. PRIMITIVUS* NEMATODE

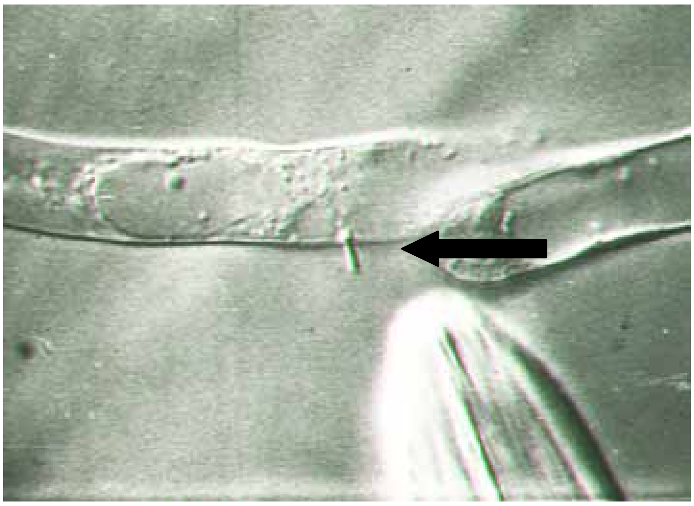

FIGURE 3B NEMATODE FEEDING NEAR ROOT CAP

Symptoms-Pathogenicity

Feeding causes root tips to stop growing and appear “stubby” or “fanged”.

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Importance

Stubby root nematode is widely distributed and can cause damage on many crops. It is often found in fields that have other species of plant parasitic nematodes. Multiple nematode species in a single field increase the difficulty of developing resistant plant varieties because resistance genes are usually effective against single genera or several species within a single genus. It is injurious to carrots in Europe, United States and elsewhere and to tomato and onion in the south eastern U.S. The transmission of Tobacco Rattle Virus causes corky ringspot / spraing symptoms in a number of commercially important potato varieties.

TABLE 2 INITIAL GROUPING OF SOME UK CULTIVARS.

Group 1 represent those varieties with known resistance to TRV and do not exhibit spraing symptoms. Group 2 represents those cultivars which exhibit classic and severe spraing symptoms eg distinct necrotic arcs in tuber flesh and do not as a rule result in systemic infections. Group 3 varieties exhibit limited spraing symptoms in the tuber flesh-fewer less distinct necrotic arcs or necrotic marks in the tuber flesh but often exhibit (sometimes transient) symptoms in the foliage and become systemically infected and can remain infected with the virus over many tuber generations across years and can act as a virus reservoir when transferred to new soils / fields / farms which harbour the appropriate trichodorid vector nematodes.

Group1: **Resistant**		Group 2: **Spraing sensitive**		Group 3: **TRV Susceptible**	
Arran Pilot	(9)	Pentland Dell	(1)	Wilja	(5)
Bintje	(7)	Maris Bard	(2)	King Edward	(6)
Record	(9)	Picasso	(1)	Santé	(6)
Saturna	(7)	Russet Burbank	(2)	Arran Consul	(6)
Climax				Nadine	(6)
Nicola	(8)			Shepody	(6)
Lady Rosetta	(8)			Saxon	(7)
Fianna	(8)			Marfona	(6)
				Rocket	(5)
				Home Guard	(7)

Characteristics

Trichodorus - Female body stout 0.5-1.4 mm long, **cigar-shaped** especially when killed by heat. Cuticle thin and rather loose without obvious annulation or lateral fields. **Mouth stylet an elongated ventrally curved tooth**; narrow anterior esophagus gradually expands into a glandular, spatulate, posterior bulb which abuts the intestine. **Vulva usually median with strong vaginal sclerotization and musculature**, gonads paired and opposed with reflexed ovaries; **tail usually rounded, very short, the anus almost terminal.** Males with elongate, ventrally curved spicules and a gubernaculum about a third the spicule length; **bursa absent but three prominent ventro-median papillae** opposite and anterior to the spicules; **tail curls ventrally when killed by heat.** Male esophageal region similar to female but with **one to four prominent ventral papillae in the onchiostyle region** in addition to the excretory pore.

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Paratrichodorus - Similar to *Trichodorus* but cuticle somewhat looser and swells more in acid fixatives. Posterior esophagus may overlap the intestine or intestine may extend forward over the basal bulb. Vaginal sclerotization and musculature much weaker than in *Trichodorus*. Males usually straight when killed by heat, **bursa present**, spicular and copulatory muscles much weaker than in *Trichodorus*.

Distribution of *Trichodorus* spp. nematodes

The nematodes are widely distributed in temperate to sub-tropical climates. Found in Europe, United States of America, Japan and China. There are 11 species known in the UK.

Within the UK, a joint study of the distribution of nematodes, including the Trichodoridae, was undertaken by Scottish Crop Research Institute, Dundee and the Institute of Terrestrial Ecology, Abbots Ripton in 1977. The maps are presented here derived from some 4600 records, supplemented by data collected at SCRI, SAC and CSL since then. The maps illustrate the distribution patterns for the species – collectively overall and also for the individual species and provide information on the relative incidence of these economically important nematodes, with points representing known infestations within 10km square areas. (based on Heath, *et al*, 1977). The accuracy of the distributions vary slightly within the UK regions, with the Scottish area being most intensively sampled, with 95% of the 10km grid squares being sampled. However the distributions presented for other areas of the UK may not be so comprehensive, such that some areas may not have been sampled sufficiently intensively (10 km squre areas) and so may not be represented by a ‘dot’. The distributions are presented in Figures 4a,4b,4c and 4d.

The important nematodes with regards to potatoes in the UK and known association as vectors for Tobacco Rattle Virus (causal virus of corky ring spot / spraing) include *Trichodorus primitivus, Paratrichodorus anemones* and *P. pachydermus*.

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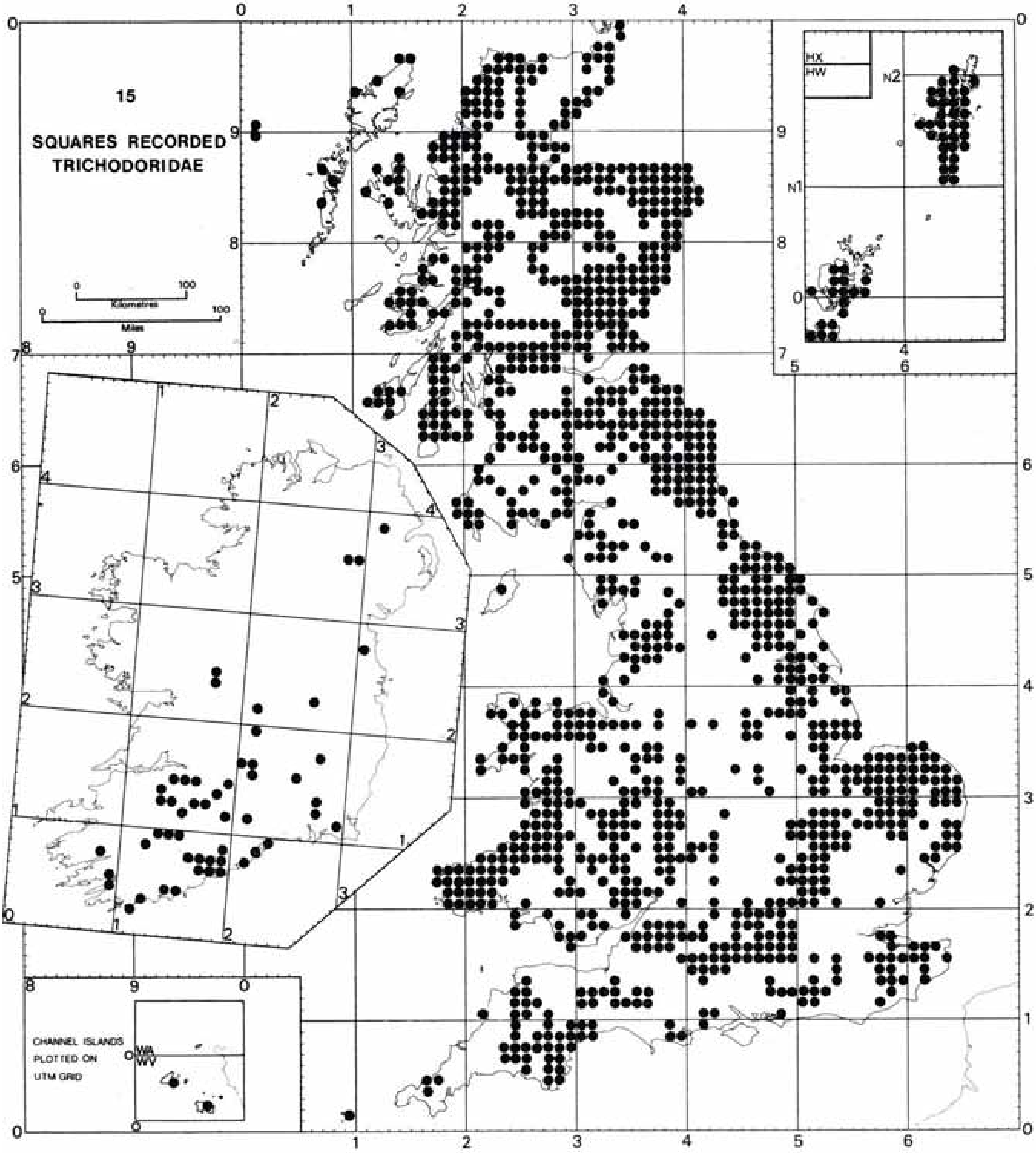

FIGURE 4A UK DISTRIBUTION OF TRICHODORID NEMATODES

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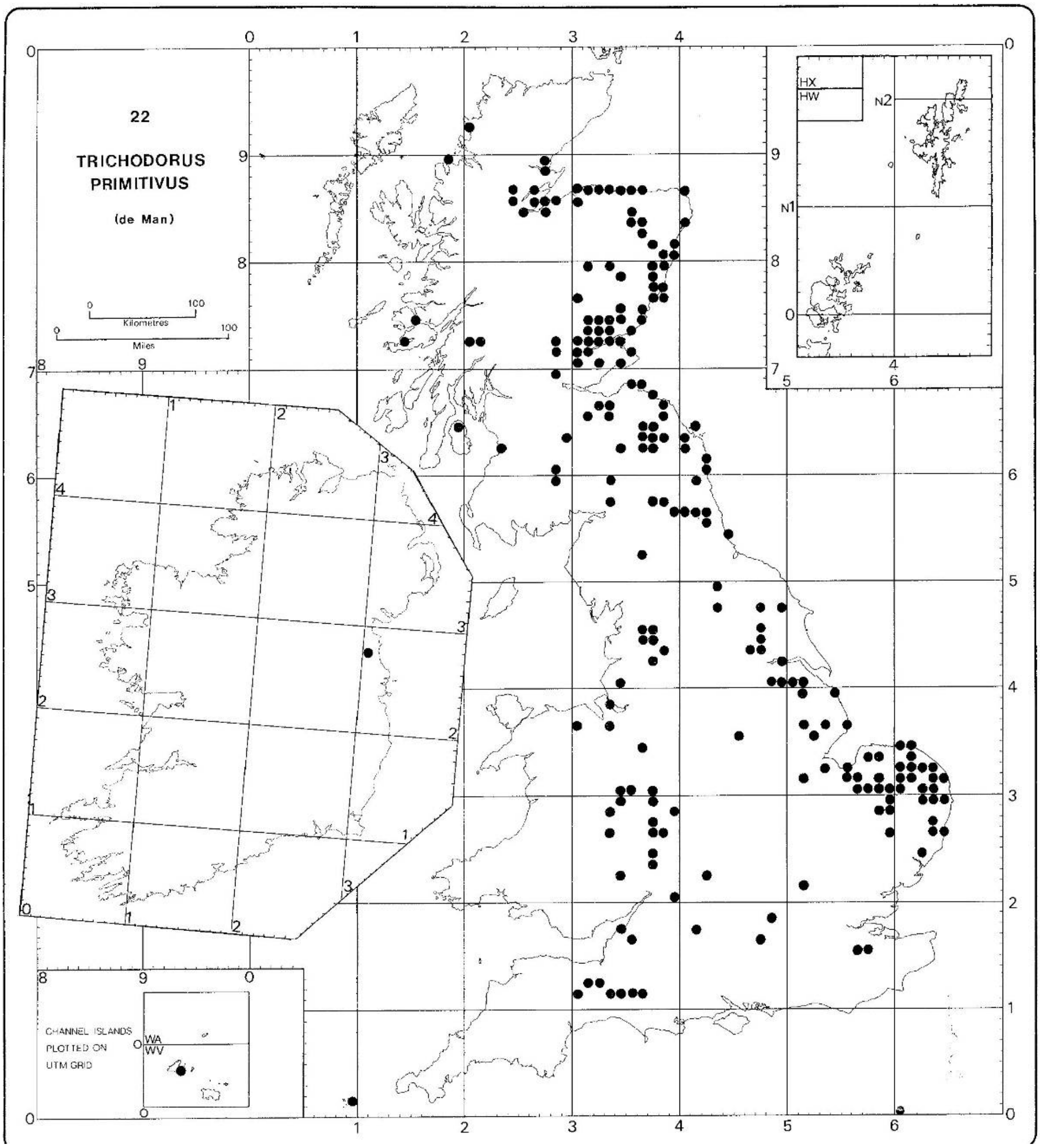

FIGURE 4B UK DISTIBUTION OF *T. PRIMITIVUS* NEMATODES

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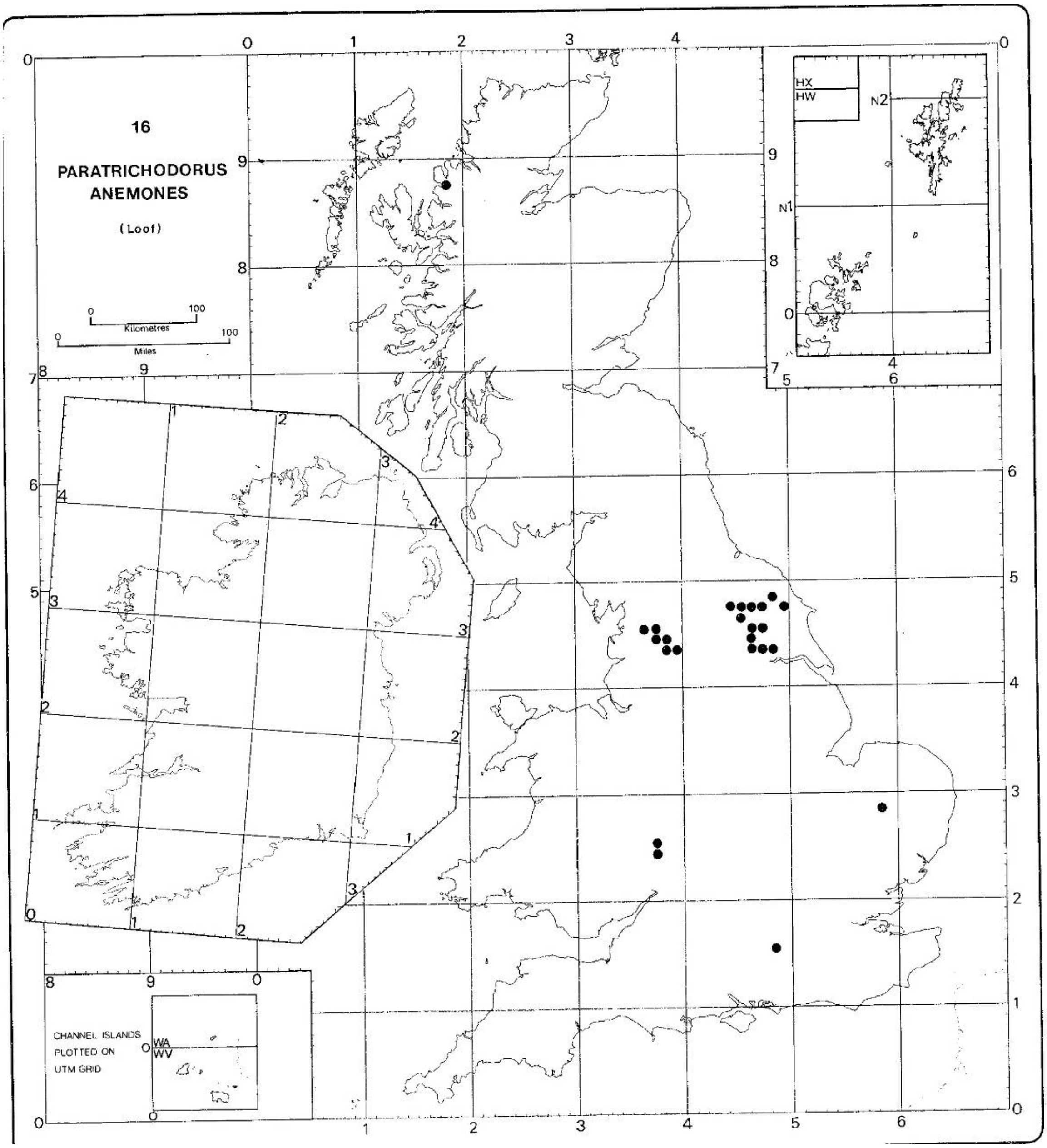

FIGURE 4C UK DISTIBUTION OF *P. ANEMONES* NEMATODES.

P. anemones has a relatively restricted distribution within the Vale of York & Lancaster areas.

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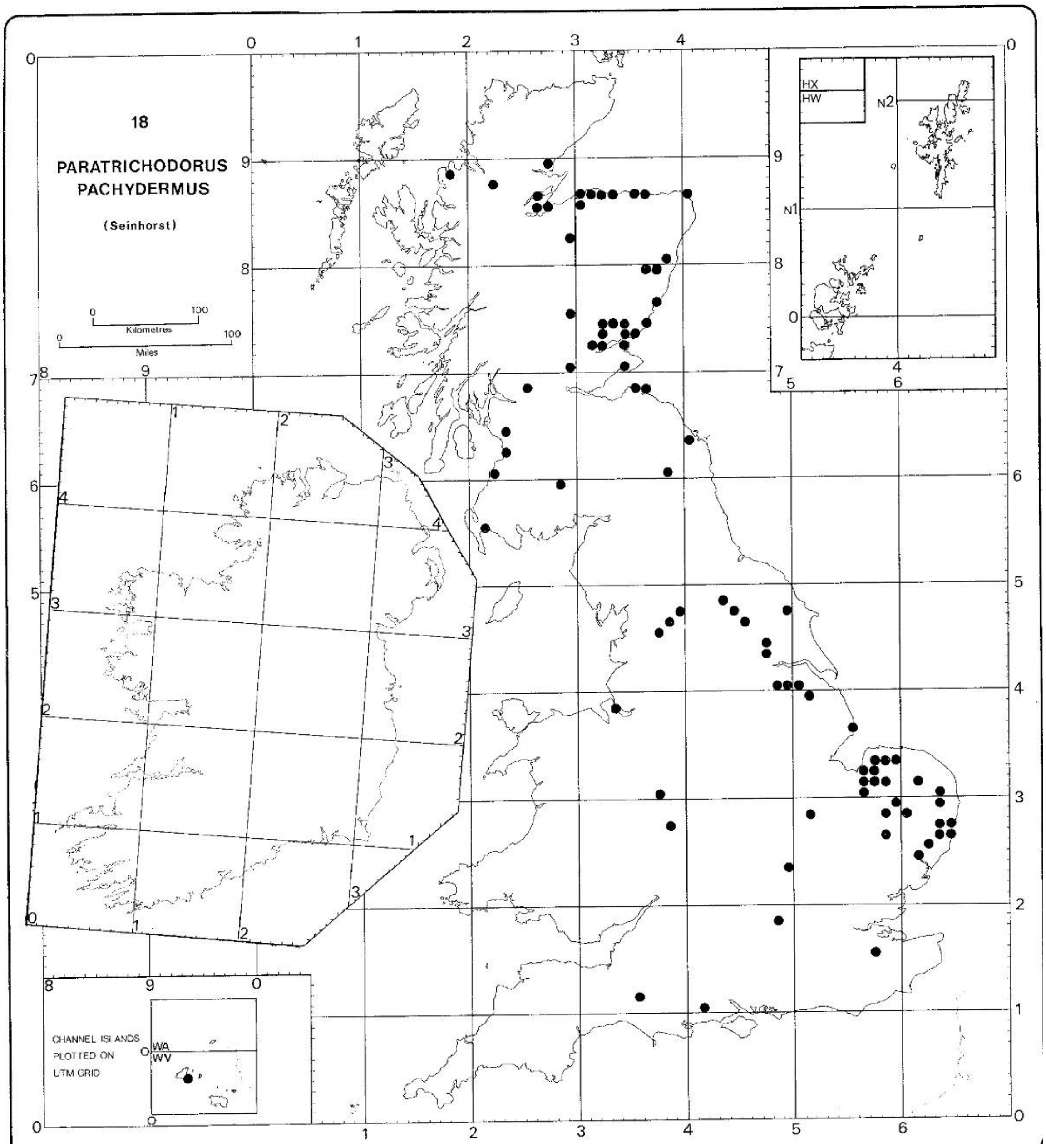

FIGURE 4D UK DISTRIBUTION OF *P. PACHYDERMUS* NEMATODES

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Free Living Nematodes – Longidorids

Longidorus elongatus
Family: Longidoridae

Longidorus macrosoma
Family: Longidoridae
Common name: needle nematode

Longidorus spp

Needle nematodes feed on a wide range of woody and herbaceous plants, attacking the root tips, which may become galled as a result. Crops that are at risk from needle nematodes, through direct feeding damage and/or nematode-vectored viruses include carrots, grasses, sugar beet, raspberry, strawberry and cereals. Damage to sugar beet (docking disorder), carrots and parsnips ("fanging") are the most notable problems associated with needle nematodes, although if numbers are high enough (> 100 nematodes/litre soil) then other crops could be at risk. 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. (www.bayercropscience.co.uk/pdfs/NematodesGuide.pdf) Needle nematodes are important vectors of raspberry ringspot virus and tomato blackring virus, and routine assessment should be carried out by raspberry and strawberry growers.

The primary needle nematode present in the UK is *Longidorus elongates*. *Longidorus macrosoma* appears to have a more restricted distribution within England. There is no clear evidence regarding number of hectares with population levels at sufficiently high levels to result in crop damage and resultant economic yield losses.

Life Cycle

Needle nematodes feed on a wide range of woody and herbaceous plants, attaching the root tips. Little information is available on yield loss in potatoes directly attributable to *L. elongatus* in potatoes (see Figure 2). Direct damage is observed in root crops such as carrot, parsnip, sugar beet (e.g. fanging, docking disorder), although yield losses in potatoes may occur at higher population levels (e.g. > 70 to 100 nematodes/litre soil). This species tends to be asexual and multiplication tends to be low on most crops (2-4 fold). However, the nematodes can live for over a year. Life cycle is illustrated in Figure 5.

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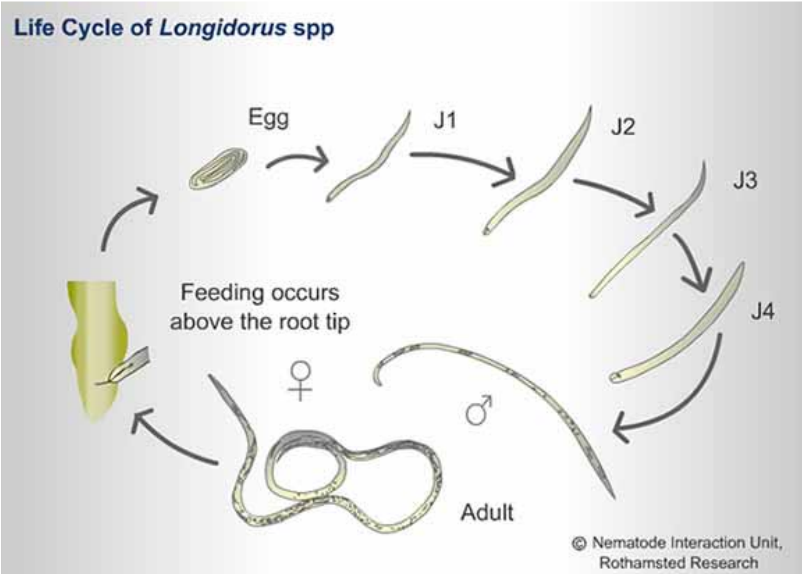

FIGURE 5
HTTP://WWW.ROTHAMSTED.AC.UK/PPI/PCNCONTROL/LONGLIFECYCLEANIM.HTM

Distribution

Longidorid nematodes are found in temperate regions; Britain, Europe, Canada, New Zealand, and U.S. For example, *Longidorus elongatus* is prevalent in lighter soils in Scotland and NE England (Figure 6.a), while *Longidorus macrosoma* is found on heavier soils in southern England (Figure 6.b). While distribution of the nematodes are available, there is no data regarding areas or hectares affected within the UK, or the crop losses or costs to growers.

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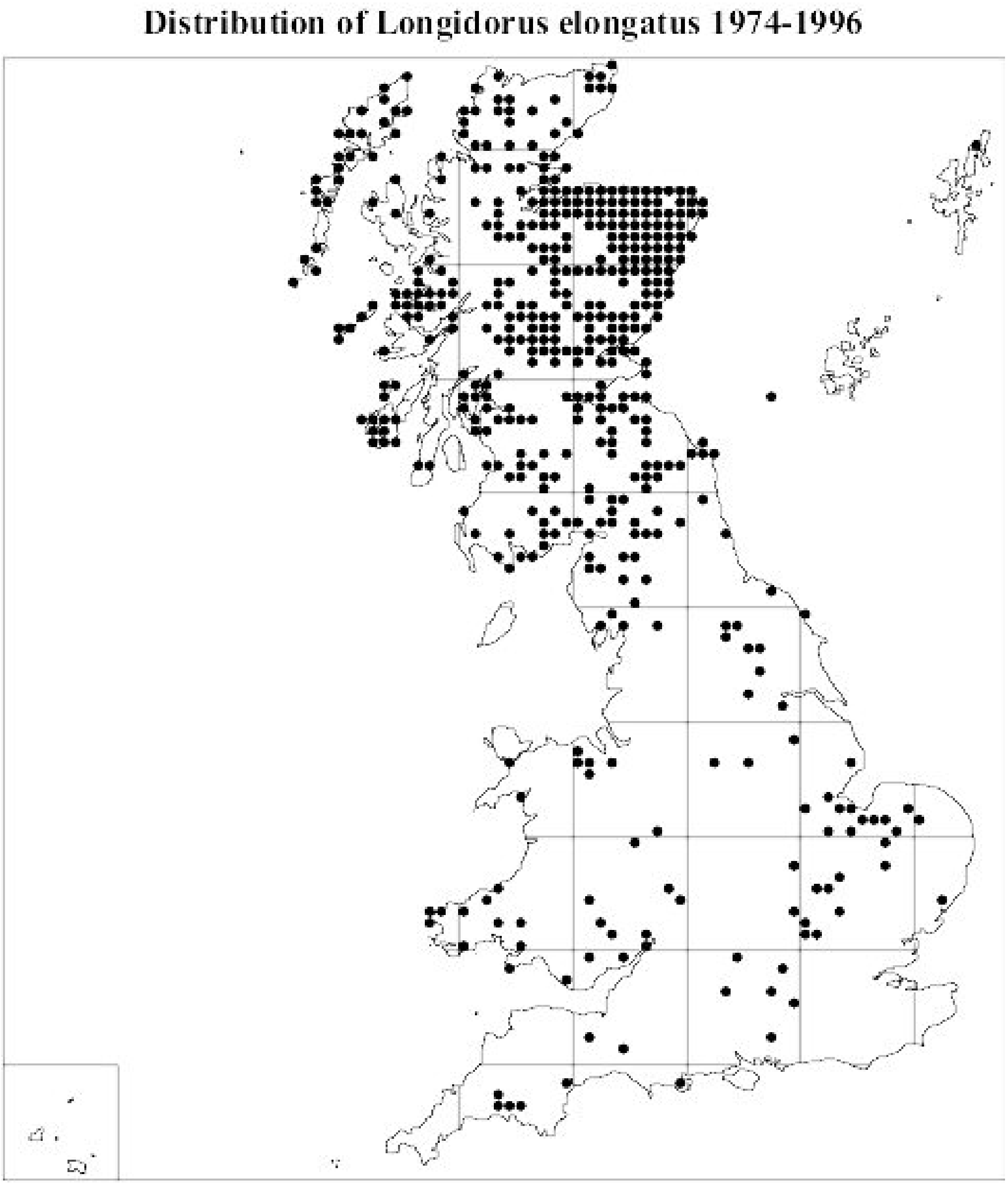

FIGURE 6A UK DISTRIBUTION OF *LONGIDORUS ELONGATUS* NEMATODES 1974-1996

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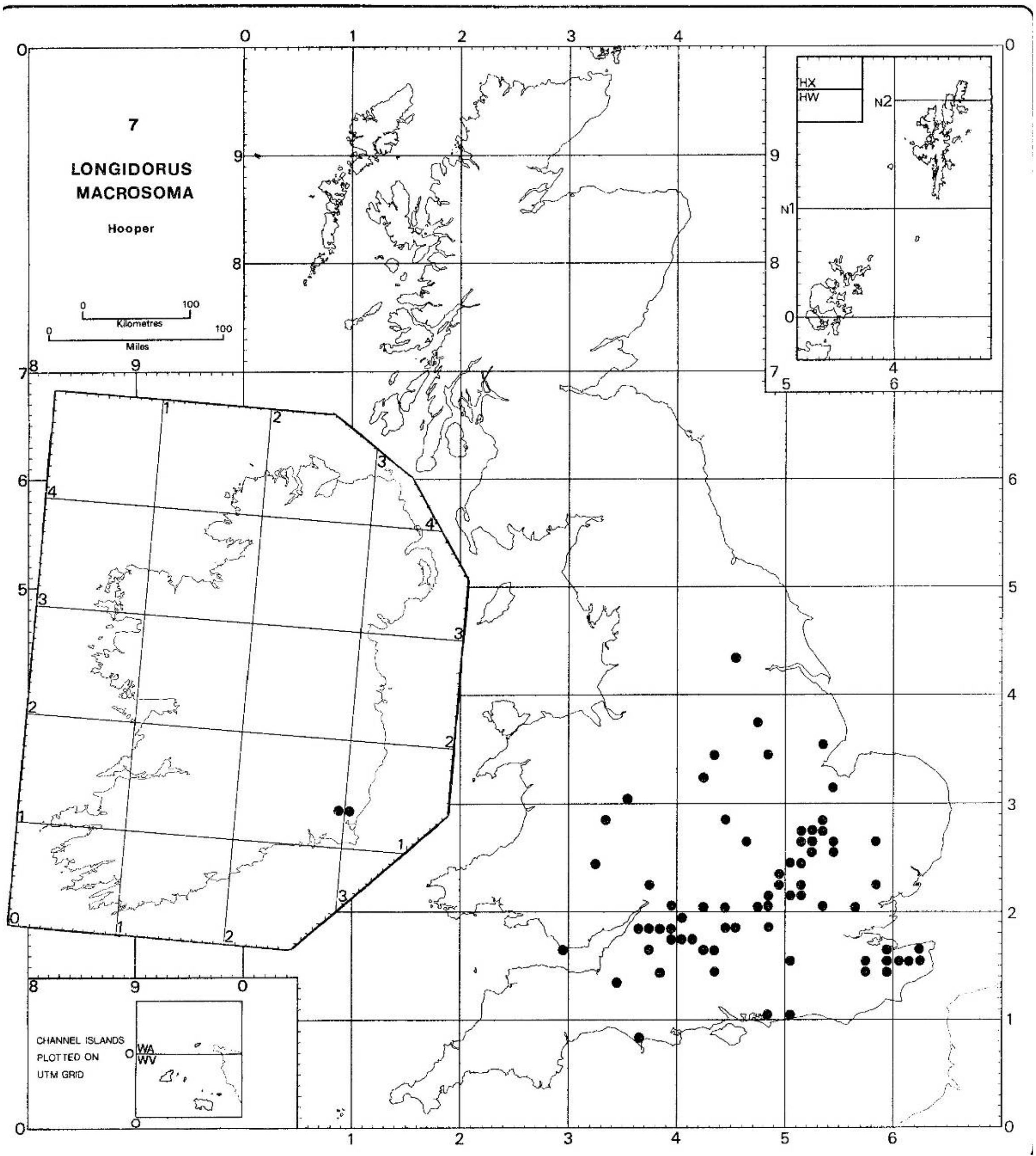

FIGURE 6B UK DISTRIBUTION OF *LONGIDORUS MACROSOMA* NEMATODES 1974-1996

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Free Living Nematodes – *Pratylenchus* spp.

Family: **Pratylenchidae**
Common name: **root lesion nematode**

The genus *Pratylenchus* currently comprises of 41 putative species which are differentiated by minute taxonomic detail, the main taxonomic character being the number of head annules. These head annules are extremely difficult to view and even under oil immersion light microscopy it is a difficult task. Consequently, electron microscopy is commonly used for unequivocal characterization of *Pratylenchus* species. Coupled with the lack of an appropriate skill-base a definitive species identification is thus rare. By default, if specimens of *Pratylenchus* are recorded in a sample by default it is assumed by many to be either *P. crenatus* or *P. penetrans* completing ignoring the more common species such as *P. pratensis*, *P. fallax* or *P. vulnus*. In this review, information was provided by stakeholders under the heading "*Pratylenchus*". We have no *a priori* information to assess whether the data provided refers to *P. crenatus* or *P. penetrans*. Therefore for the purposes of this review we use the general descriptor, *Pratylenchus*.

Pratylenchus remains thread-like and mobile during its whole life-cycle. Approximately 400 different crop and weed species serve as hosts. This broad host range minimizes the potential to manage these nematodes by crop rotation with non susceptible hosts. Once *Pratylenchus* has been introduced to a suitable site or field through soil movement, eg on farm implements, it is likely to persist as it has such a wide host range.

Pratylenchus penetrans (Figure 7) is one of the most common root-lesion nematode in the UK, attacking many plant species, including bulbs, ornamentals, roses, fruit trees, raspberry, strawberry, potato, tomato, carrot and lettuce. The nematodes initially feed on the outer root surface and root hairs before penetrating the roots. Population levels above c.2500 nematodes/litre soil may result in observed adverse growth in potatoes. (see Figure 2) (http://www.bayercropscience.co.uk/pdfs/NematodesGuide.pdf).

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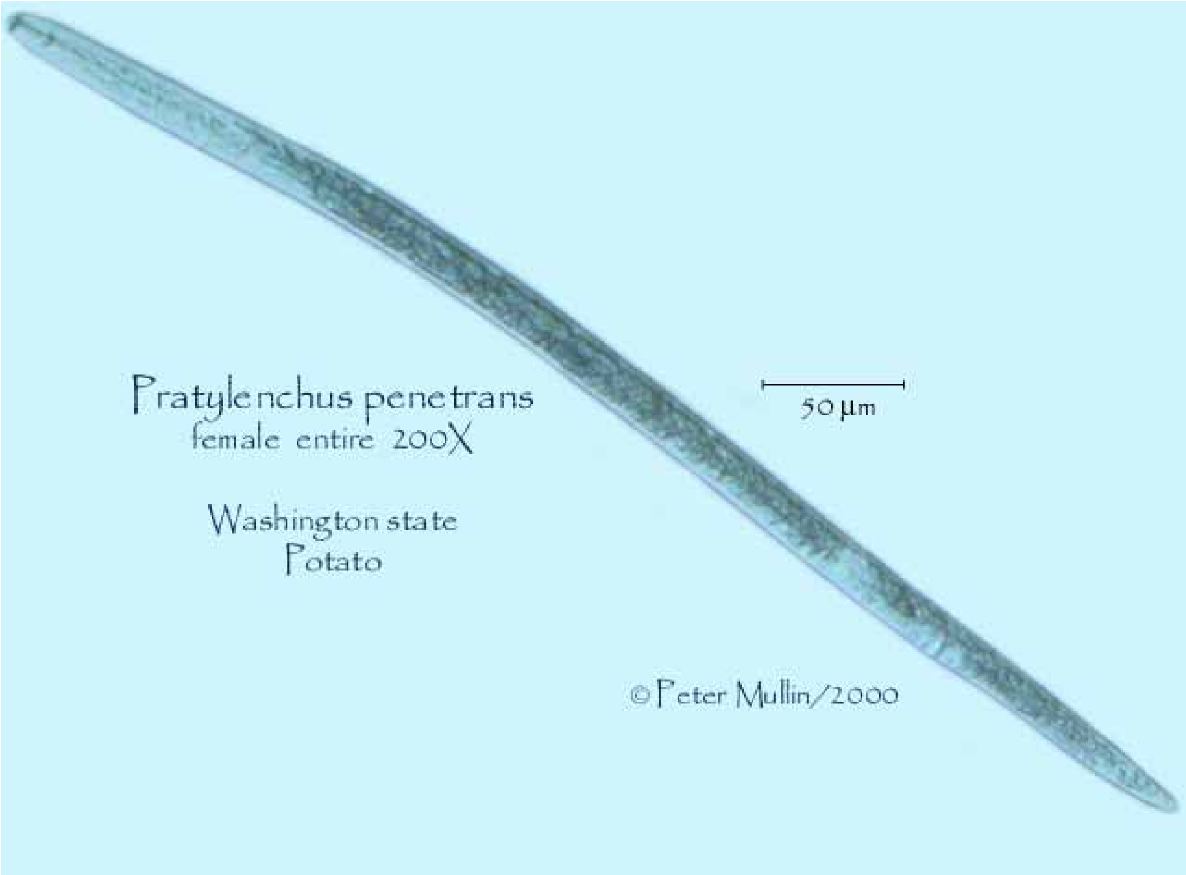

FIGURE 7 PRATYLENCHUS PENETRANS (MULLIN, 2000)

Biology

P. penetrans is one of the two species of Pratylenchus which are particularly harmful, the other one being *P. vulnus*. Both nematodes parasitize almost all kinds of fruit tree, including ***peach***, ***almond***, ***apple***, certain ***Citrus*** and ***cereals***, floral plants, market garden plants, ***potato*** and ***vine*** . However, unlike *P. vulnus*, *P. penetrans* does not attack ***walnut***, ***fig*** or ***olive*** but may be harmful to ***hazel***. It is an important parasite of the roots of various ***meadow Gramineae*** in oceanic climates. In Holland, it causes severe ***damage*** to pasture land, hence the name meadow nematode. Conversely, *P. vulnus* has a preference for sandy soils in the South. The nematodes remain vermiform and motile throughout their larval and adult stages, and all stages from L2 on can infect plants by penetrating the root and invading the cortex. These nematodes attack the external cells of radicles and penetrate the tissues little by little whatever the stage of development. They create cavities in which they reproduce and tissues may contain thousands of individuals.

They gradually make their way to parts of the parenchyma that are still sound, affected areas being rapidly destroyed by a characteristic necrosis. When environmental conditions are unfavourable, e.g. the roots decompose, nematodes leave the root and travel freely within the soil until they come across another host root.

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Crop damage

Pratylenchus causes the formation of deep necroses in the roots, providing entry for and encouraging attacks by secondary parasites, especially fungi, which infect the plant and finally mask the responsibility of the nematode for the observed fall in yield. Necroses appear on the roots of potato and pustules form on the tubers, whose value is then reduced. Maximum damage occurs in sandy soils, since these nematodes prefer this type of soil, and also because sandy soils are often more suited for potato production. Above-ground symptoms caused by high populations of root lesion nematodes are sometimes wrongly attributed to lack of water or nutrients.

The main entry points are the regions of root hair development (Zunke 1990) and the elongation zone (Townshend 1978). *P. penetrans* migrates intracellularly by puncturing cell walls with its stylet and may feed briefly before invading a cell. In addition to mechanical force generated through its stylet and body (Zunke 1990), *P. penetrans* also appears to degrade cell walls by secreting cellulolytic enzymes (Krusberg 1960; Uehara *et al.,* 2001). During typical feeding periods (5–10 min) a salivation zone develops at the stylet tip and cytoplasmic streaming increases in the plant cell, but the affected cell does not die (Zunke 1990b).

On potatoes, the *Globodera–Verticillium dahliae* and *Pratylenchus–Verticillium dahliae* disease complexes have been reported. Early senescence or 'early dying' caused by *V. dahliae* and *V. albo-atrum* is accentuated by populations of *Pratylenchus* spp. (Martin *et al*., 1981; Wheeler *et al*., 1992; Bowers *et al*., 1996; Hafez *et al*., 1999), *G. rostochiensis* (Evans, 1987), and *G. pallida* (Hide *et al*., 1984; Storey & Evans, 1987). In terms of yield, Martin *et al*. (1982) calculated that 15, 50 and 150 *P. penetrans* per 100 cm^3 soil in combination with *V. dahliae* would result in 36, 60 and 75% reductions in potato tuber weight, respectively. However, tuber weights were unaffected by the presence of the individual pathogens, except where nematode populations were high (150 *P. penetrans* per 100 cm^3), when a 12% reduction was found. Yield reduction from the *Pratylenchus–V. dahliae* complex has also been reported (Botseas & Rowe, 1994), as have other damaging effects such as the disruption of photosynthesis, stomatal conduction and transpiration (Saeed *et al*., 1997a; Saeed *et al*., 1997b). However, the fundamental importance of *P. penetrans* in potato early dying is its ability to activate low populations of *V. dahliae* that would otherwise be inconsequential in disease development (Bowers *et al*., 1996).

Life Cycle

The duration of the life-cycle may vary from 1 to 2 months and several generations may succeed one another (Figure 8), probably influenced by environmental factors such as soil moisture content and soil temperatures. Sexually reproducing, males and females present with eggs deposited in roots and soil. As the *Pratylenchus* female progresses through the soil and roots she deposits one or two eggs per day along the way. Several generations are possible within one season.

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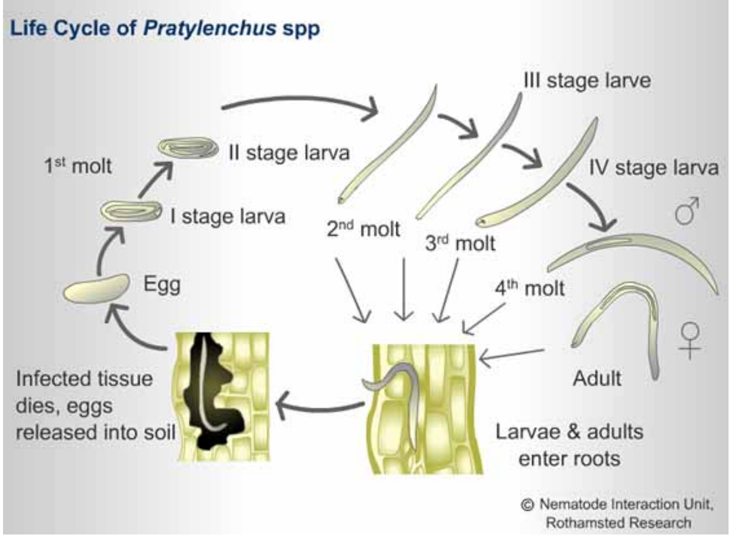

FIGURE 8 LIFE CYCLE OF *PRATYLENCHUS* SPECIES OF NEMATODE.
http://www.rothamsted.ac.uk/ppi/pcncontrol/longlifecycleanim.htm

Distribution

Pratylenchus are widely dispersed, mainly in temperate regions, sandy soils and are disseminated by transportation of soil or plant parts and by surface or irrigation water (Evans *et al*. 1993). While distribution of the nematodes are available, there is no data regarding areas or hectares affected within the UK, or the crop losses or costs to growers.

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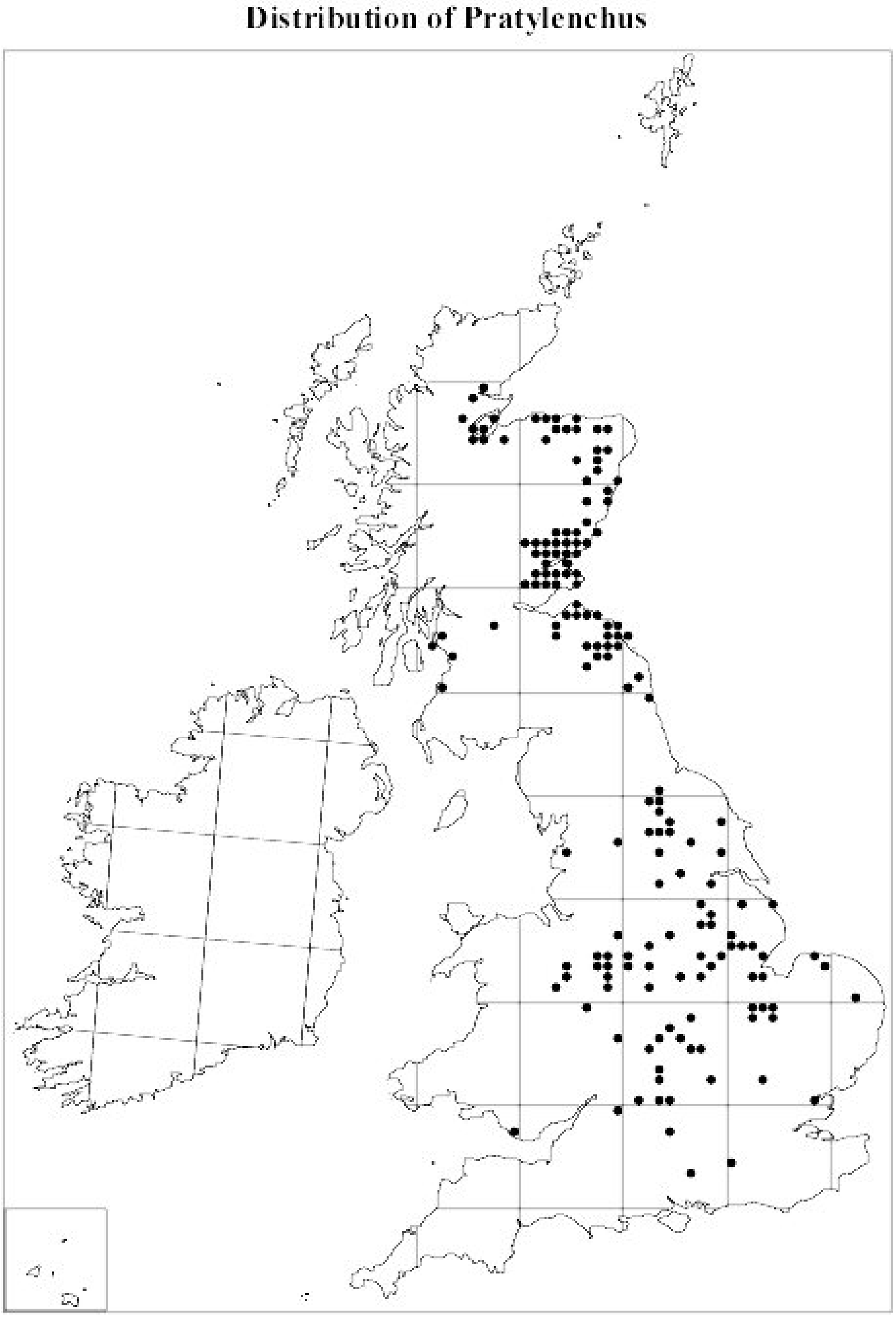

FIGURE 9 UK DISTRIBUTION OF *PRATYLENCHUS SSP.* NEMATODES 1974-1996

Yield losses due to *P. penetrans. P. penetrans* damage threshold densities for potato was established at 100 to 200 nematodes 100 cm^{-3} soil (Olthof, 1987). In addition, the nematodes interact synergistically with *Verticillium dahliae* (Verticillium wilt) to further reduce potato growth and yield (Bowers et al., 1996; Saeed et al., 1998). Nematode densities alone were not highly correlated with potato yields, soil moisture, or soil pH but were consistently correlated with Verticillium wilt (*Verticillium dahliae* Kleb.) symptoms.

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Cultural practices such as multiyear cropping regimes (Chen *et al*., 1995) and soil solarization (Pinkerton *et al*., 2000) have had variable success in reducing population densities of *V. dahliae* and *P. penetrans*. Pinkerton *et al* found that soil solarization, cover crops plus solarization, or fumigation with metam sodium reduced ($P < 0.05$) *V. dahliae* at 5 and 10 cm. In greenhouse assays of solarized soils, disease severity was reduced ($P < 0.05$) for *Verticillium* spp. on eggplant and *Phytophthora* spp. on snapdragons. Cover crops alone were not effective in reducing *P. cinnamomi* and *V. dahliae* populations. The authors reported that their research based in Oregon, with high temperatures, demonstrated that solarization can reduce, but not eliminate, population densities of *Pratylenchus penetrans* in the upper 30-cm soil profile. This effect agrees with other reports showing that population densities of *Pratylenchus* spp. were reduced 50 to 100% to soil depths of 10 to 15 cm by solarization, but were not significantly reduced at depths below 15 cm. However, such high soil temperatures – under plastic up to 53°C (5cm deep) to 34°C (30cm deep) within the UK over a c.40 day period may be difficult to achieve.

While distribution of the nematodes are available, there is no data regarding areas or hectares affected within the UK, or the crop losses or costs to growers.

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Distribution of Tobacco Rattle Virus

Introduction

Tobacco rattle virus (TRV) is a *Tobravirus* which causes corky ringspot or spraing disease. This virus is transmitted by 'stubby-root' nematodes in the *Paratrichodorus* or *Trichodorus* genus, and can also be transmitted mechanically. It occurs more often in coarse sandy soils. Symptoms in virus susceptible varieties appear on the foliage with some symptoms in the tuber. However the tubers of spraing susceptible varieties contain corky layers of tissue interspersed with rings of healthy tissue and brown flecks distributed throughout the tuber.

Tobacco rattle virus (TRV) can cause a number of different symptoms in potato plants including necrotic arcing (known as spraing, corky ringspot) in the tuber flesh, and stem-mottle (distortion, stunting and mottling) and aucuba in the foliage. TRV has a wide host range, particularly among weed species, potentially infecting more than 400 monocotyledonous and dicotyledonous species across over 50 families. The virus does not usually become systemic in most of these hosts, often remaining in the roots. However, several species can become systemically infected and some, such as *Stellaria media*, may show no obvious foliar symptoms. The virus host range includes many common agricultural weed species (Cooper *et al*, 1971), and these weeds serve to perpetuate the virus within sites and the nematode populations. Distribution of the virus in the soil reflects that of its free living nematode vectors, which prefer light and/or sandy soils. Potato cultivars differ in their propensity to develop spraing symptoms when exposed to viruliferous nematodes; some, such as Pentland Dell and Maris Bard, are highly susceptible whereas others, such as Record, are resistant to infection by TRV. However, Xenophontos *et al.* (1998) and Dale *et al.* (2000) demonstrated that some cultivars, such as Wilja, that rarely exhibit spraing symptoms can become infected with TRV without developing spraing symptoms. This type of infection differs in several ways from that in spraing-susceptible cultivars. Thus, infections associated with spraing symptoms tend not to become fully systemic and are transmitted to only a proportion of daughter tubers. In contrast, TRV infections in cultivars that exhibit few, if any, spraing symptoms tend to be transmitted to all daughter tubers and can persist through many vegetative generations with virus recoverable from all parts of the plants. Moreover, such plants can serve as sources for virus acquisition by vector nematodes (Xenophontos *et al.,* 1998) and hence serve to distribute the virus over considerable distances to new sites, if the appropriate nematodes are present (Dale *et al.*, 2000).

Few satisfactory methods are available for the control of TRV infection, and the situation will become worse with the imminent withdrawal of aldicarb, one of the principle chemicals used to control the activity of the trichodorid nematodes that transmit the virus.

Of the approximately ninety species that represent the genera *Trichodorus* and *Paratrichodorus*, only thirteen are known to transmit three tobraviruses, namely *Tobacco rattle virus* (TRV), *Pea early-browning virus* (PEBV) and *Pepper ringspot virus* (PRV), to a range of agricultural crops (Taylor and Brown, 1997). Many of these vector nematode species occur as mixed populations including non-vector species from

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the same genera (Boag and Topham, 1985; Topham *et al*., 1985), with instances when only one species within mixed populations being viruliferous.

There are a number of different species of Trichodorid and Paratrichodorid nematodes found in the U.K. and in Europe, with different strains of the virus associated with different species. In the United Kingdom and Germany, The FLNs *T. primitivus* and *P. pachydermus* and their associated RQ and PRN strains of TRV, respectively, are the most frequently found trichodorid vector and virus combinations in potato fields. The associations are presented in Table 3.

TABLE 3 ASSOCIATIONS BETWEEN *PARATRICHODORUS* AND *TRICHODORUS* SPECIES AND SEROLOGICALLY DISTINGUISHABLE STRAINS OF *PEA EARLY-BROWNING* (PEBV), *PEPPER RINGSPOT* (PRV), AND *TOBACCO RATTLE* (TRV) TOBRAVIRUSES.

Nematode	Tobravirus	Strain
P. allius	TRV	USA
P. anemones	PEBV	English
	TRV	PaY4
P. minor	PRV	Brazil
	TRV	USA
P. nanus	TRV	PRN
P. pachydermus	PEBV	Dutch
	TRV	PRN (PpK20)
		PaY4
P. teres	PEBV	Dutch
	TRV	Oregon
P. tunisiensis	TRV	Italian
T. cylindricus	PEBV	English
	TRV	RQ
		TcB28
T. primitivus	PEBV	TpA56
	TRV	TpO1
T. similis	TRV	Ts-Belgian
		Ts -Dutch
		Ts-Greek
T. viruliferus	PEBV	English
	TRV	RQ

Distributions viruliferous nematode populations.

Information on the distribution of viruliferous nematode populations within the UK is extremely patchy. This study has gathered information collected over the past 10 years by the principal researchers and advisors working in the industry, including CSL at York, SAC based in Edinburgh and SCRI at Dundee, with further information from limited questionnaire returns from growers and industry representatives and also limited information from the chemical/nematicide industry. Further information was collated from the limited publications and articles in this area.

Different estimates are available regarding the proportions of Trichodorid and Paratrichodorid nematode populations that appear viruliferous. Parker (ADAS, 2005) estimated that in Scotland, the nematodes were in 65% of lighter, or sandier, soils and

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were in up to c.33% of lighter soils in England, of which 80% were believed to be carrying the virus. K.A. Evans (2006) gave the following figures, presented in Table 4.

TABLE 4 RESULTS FROM LOW SAMPLE NUMBERS (<10). FROM EVANS 2006.

Region	% of TRV positive samples	As % contribution of total samples examined
Lothians	35	6
Borders	27	10
Fife	23	27
Perthshire/Angus/Tayside	21	48
Aberdeenshire	29*	3
Elgin/Inverness	78*	3
Ayrshire/Dumfries	75*	3

The author estimated that 30% of field were viruliferous, based on 311 samples. Evans estimated 30 to 35% for soil samples from Lothians, Borders and Fife, with lower proportions from Perthshire and elsewhere, though sampling will have been affected by advisor/grower knowledge targeting known suspect areas in both cases.

The earlier reports of the distribution of the virus were confirmed to the authors through the data collected from the various agencies and individuals, with the principal 'hotspots' focused largely as expected in areas of lighter soils, including in Scotland the areas sourrounding Elgin, the Angus area around Forfar – Brechin in the Vale of Strathmore, parts of Perthshire, through Fife and into the East Lothian area.

CSL began offering a spraing testing service in the late 1990s (1998-99). Hence prior to then, there is very little data available regarding TRV distribution from its testing service. However, given the fact that so many of the samples received at CSL come via third party sources, in particular national companies and other laboratories, working out which regions/counties samples actually come from has proved extremely difficult and this applies to much of the soil diagnostics / testing carried out in the UK.

TABLE 5 CSL RESULTS REGARDING DISTRIBUTION OF TRV BASED ON CSL SOIL TESTING.

Region	% of total *	Comments
Yorkshire	40	Virtually all from North Yorkshire
Scotland	18	
East Anglia	17	Evenly distributed between Norfolk, Lincolnshire and Cambridgeshire
West Midlands	8	Mainly Shropshire
Others	All <5	North West (Lancashire); East Midlands (Notts and Leicestershire); South (Sussex, Isle of Wight); Isle of Man
Others	0	South West of England

*Based on results from 60 samples received from 1998 to the present, **where origin was confirmed**. This includes both tuber and soil samples.

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NOTE: sub-comparisons between samples from 98-99 and 04-06 confirm relative proportions, with very little differences between the 4 key areas listed.

TRV incidence in samples showing spraing symptoms

Diagnosis	% of total *
TRV	51
PMTV	18
Negative	31

* Based on results from 109 samples received from 2002 to the present.
NOTE: 2 of these samples were mixed infections of both TRV and PMTV.

TRV incidence in soil samples

Diagnosis	% of total *
TRV positive	19
TRV negative	81

* Based on results from 666 samples received between 2003 and the present

Overall, CSL identified c.19% TRV positive from a total of 666 soil samples tested. The CSL data has been incorporated into the TRV distribution map.

Assessment of SCRI records (Neilson, 2006 pers comm) indicate that out of an area from Northumberland northwards, largely on the east coast of Scotland, as above, some 31% of samples were positive for TRV. Estimates using the 41% of grower returns which detailed the area affected indicated that from 159 samples, 560.6 ha out of 1288.6 hectares had tested positive for the virus.

Distribution of TRV within the UK is illustrated in Figure 10.

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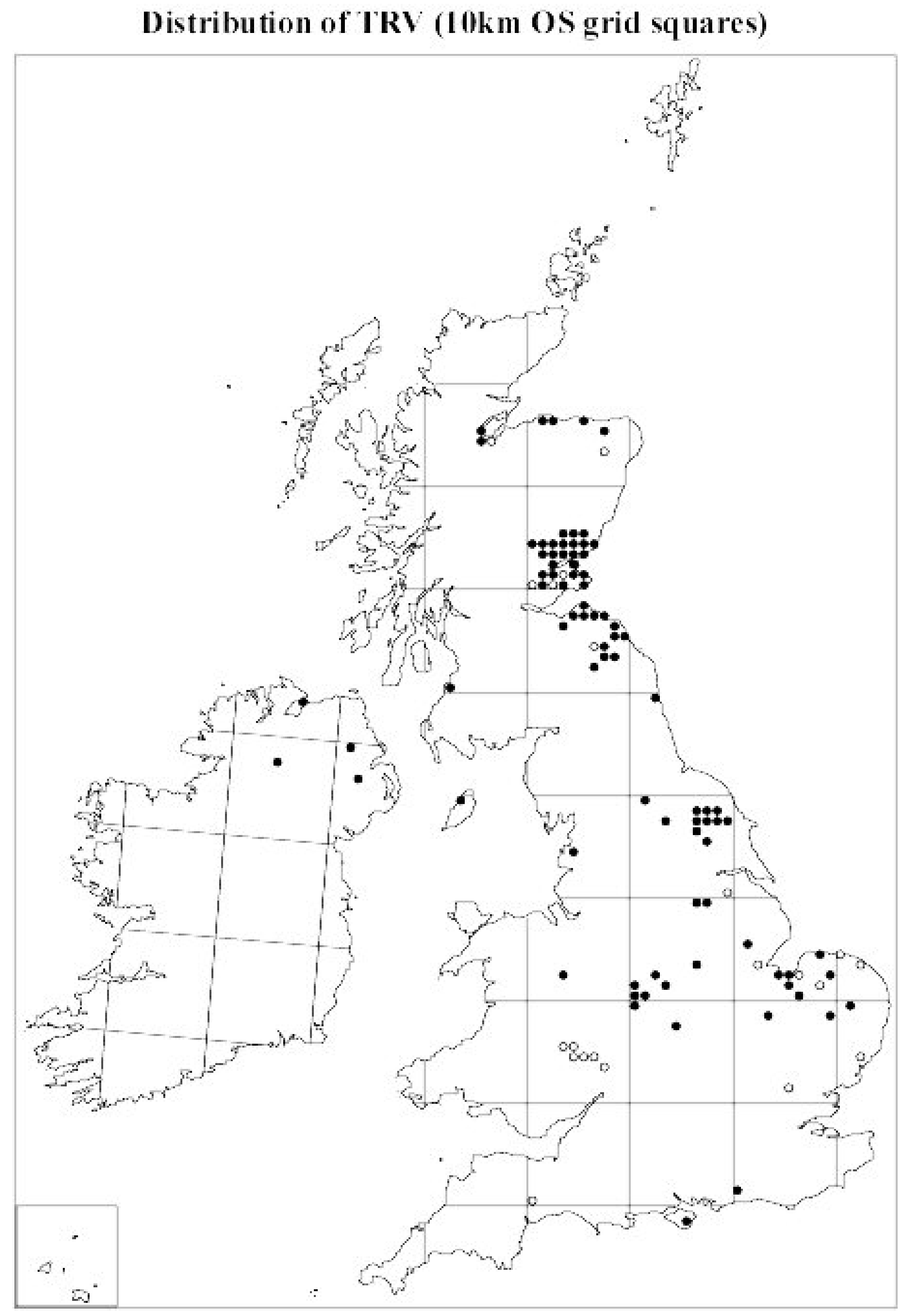

FIGURE 10 DISTRIBUTION OF TOBACCO RATTLE VIRUS / SPRAING / CORKY RING SPOT IN THE UK, BASED ON KNOWN REPORTS FROM SOIL TESTS AND REPORTS BY GROWERS OVER PERIOD 1995 TO 2006.

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In Northern Ireland, Tobacco Rattle Virus has been relatively uncommon or unreported, but has been detected in Magilligan (Co. Londonderry), Maze and Drumbeg (Co. Antrim), and Sandhill (Co. Tyrone). In the last few years spraing caused by TRV has also been found in several parts of Co. Down. No details were available regarding the extent of the areas affected.

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Detection of Tobacco Rattle Virus

The detection and identification of trichodorids and associated Tobacco Rattle Virus until recently has been a laborious task. There is a poor relationship between nematode numbers and the risk of spraing in the potato crop. In any field population there are often mixtures of different nematodes, with perhaps only one being viruliferous. Nematodes have to be extracted from soil (Brown and Boag, 1988), individually hand-picked and either go through an approximate 4 week process to fix and mount specimens on slides for classical light microscopy based taxonomy (Decraemer and Baujard, 1998) or used in laboratory experiments to determine vector capability and efficiency (Brown et al., 1989). The detection of TRV used indicator plants in a bait test that yielded results after 4-6 weeks (Brown *et al.,* 1988; 1989).

Accurate species identification is imperative for phytosanitary and management reasons. Species delineation within the genera *Paratrichodorus* and *Trichodorus* is especially problematical as it is based on minute differences in morphological characters that frequently overlap across morphologically conservative species (Decraemer and Baujard, 1998). Problems associated with identification are further compounded, due to the continuing decline in classical taxonomic expertise (Behan-Pelletier, 1999; Andre *et al.,* 2001; Coomans, 2002). Consequently, there is an increasing reliance on the development of molecular-based diagnostic protocols for identification purposes (Jones et al., 1997; Powers *et al.,* 1997; De Giorgi *et al.,* 1999; Powers, 2004).

With few exceptions, molecular-based diagnostics developed to date for nematodes have been focussed primarily on the endoparasitic or semi-endoparasitic genera *Meloidogyne*, *Heterodera*, *Pratylenchus* and *Globodera* (for example, Zijlstra *et al.,* 1995; Shields *et al.,* 1996; Uehara *et al.,* 1998; Subbotin *et al.,* 1999, 2000). As Hübschen *et al.* (2004) noted, there is a paucity of rigorously validated nematode diagnostics, most at best, are of a confirmatory standard for internal laboratory purposes only rather than critical phytosanitary usage. Consequently, a validated reliable and rapid molecular-based pre-plant soil-test for determining the presence of vector trichodorid species is not generally available. However, such tests are essential to determine the most appropriate disease management strategies, including efficient targeting of nematicide applications. Recently three studies (Boutsika *et al*., 2004; Holeva *et al*., 2006; Riga *et al.,* 2006) have produced peer reviewed diagnostics for virus-vector trichodorid nematodes, although only Riga *et al.* (2006) followed the validation criteria established by Hübschen *et al.* (2004).

Currently NIAB offer a test for TRV based on a molecular PCR-based test, which can be performed directly on tubers or on a soil sample. The soil-based test requires using tobacco as a host bait plant, allowing the nematodes to feed on the bait plants and subsequently testing those plants for virus. The tuber test takes 5 days whilst the soil test takes 28-35 days.

NIAB also offer to test for Needle nematode (*Longidorus*), Root Lesion nematode (*Pratylenchus*): High populations cause reduced growth, yellowing and smaller tubers, and also cause lesions on both the roots and tubers. .Stem nematode (*Ditylenchus*): occasionally attacks tubers causing conical pits with skin splitting. In most cases, NIAB indicates that the degree of loss depends on the nematode species, population density and the host cultivar, along with local climatic / seasonal conditions. The NIAB tests aim to

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provide growers with the population density, and a breakdown of the FLN species present in the soil.

CSL offer a nematode identification service based on classical taxonomy as well as a peer-reviewed real-time PCR (TaqMan) assay for TRV (Mumford *et al*, 2000) and appears well used by some sectors of the industry. Much of the ‘drivers’ increasing the use of various diagnostics comes from the major supermarkets requiring suppliers and growers to justify nematicide applications due to consumer conserns and pressure. SAC (Edinburgh) also offer a TaqMan PCR diagnostic but this is targeted on identifying a limited number of potential vector nematode species and would benefit from further field validation. SCRI offer a diagnostic service for nematodes and TRV, based on classical taxonomy and bait-testing plants respectively. Although this process is lengthy compared to molecular methods it is seen as the industry standard.

Considering the potential benefits of pre-plant diagnosis of the presence of trichodorid nematodes and TRV, and the increasing demands by the industry (including supermarket and processing multinationals) to limit and justify nematicide use, soil testing is becoming increasingly important. As a result of previous BPC funding, SCRI has developed a state of the art, rapid TaqMan-based combined virus-vector trichodorid and TRV assay which in laboratory tests has been demonstrated to be very sensitive and robust. Although the methodology has been successfully subjected to peer review (Holeva *et al.*, 2006), funding has not been available for rigorous validatory testing in order to bring the diagnostic to the market place.

Further BPC funded work (report available on BPC website : R255 –“TRV - improvement of a diagnostic test to allow more precise localisation of Tobacco Rattle Virus in fields”) has identified the potential of using a number of common weeds as indicators of the presence of the virus. The results are promising in regard to identifying highly viruliferous areas – or ‘hotspots – that appear to agree with spraing symptoms in the following potato crop. However, the areas of lower population levels, or lower viruliferous numbers of nematodes, and the subsequent symptom expression are difficult to predict with accuracy and probably rely on a number of environmental factors. This problem of correlating diagnostics to subsequent symptom expression in following potato crops applies to all current diagnostic procedures and is exacerbated by the fact that c.1% to 2% infection in seed crops or 4% to 6% in some ware/processing crops may cause rejection of the crop. As such the usefulness of the weed test and the other tests available and their predictive application needs further field trialing to allow growers a degree of confidence and allow decision making with regard to management options.

Accordingly, reliable, proven and rapid pre-plant soil-test for determining the presence of TRV in association with its vector nematode species is not yet generally available. Such tests are essential to determine the most appropriate disease management strategies, including efficient targeting of nematicide applications. Considering the importance and consequences of successful, pre-plant diagnosis of the presence of trichodorid nematodes and TRV, and the increasing demands by the industry to limit and justify nematicide use, soil tests are becoming increasingly available through such agencies as advisors and chemical company representatives who generally look to the main independent research and advisory agencies as CSL at York, SAC at Edinburgh and SCRI at Dundee who offer various levels of test / assessment.

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Agency laboratories undertaking pre-crop soil tests for FLN and Tobacco Rattle Virus presence include:

SAC in Edinburgh: or see SAC adviser in your local directory
SCRI in Dundee: 01382 562731
ADAS High Mowthorpe: 01944 738646
NIAB Cambridge: 01223 276381
CSL Sand Hutton: 01904 462000
SASA, Edinburgh 0131 2448859 not soil diagnostic. Tests on tubers / foliage for TRV

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Effects of Trichidorid nematodes and TRV/spraing on production and quality.

The area of potatoes grown in the UK, together with the number of producers, has declined over the last 10 years, whereas yields have increased, due largely to the adoption of irrigation, improvements in soil management, and more effective machinery for seedbed preparation. Potato production is now in the hands of fewer but more specialised growers who are having to meet ever increasing demands for high quality potatoes, especially for blemish-free, pre-pack potatoes for retail. At the same time, as with other farm enterprises, there is increasing pressure for potatoes to be grown using integrated crop management principles (ICM), and this has led to the introduction and adoption of crop protocols and quality assurance schemes.

The effects of FLN and particularly TRV viruliferous nematodes are principally due to the internal tuber blemishes, 'spraing' or 'corky ring spot' that result in crop down grading in value or outright rejection. While this is perceived as processing industry problem, it is also important within some table/supermarket varieties and also is an important factor in the seed industry.

Notable varieties for 'spraing' symptom s include Pentland Dell, Maris Bard, Picasso, Russet Burbank, Cara and Desiree (see Table 2).

Varieties known to be susceptible to the virus and can carry systemic infections include Wilja, Sante, Nadine, Shepody, Saxon and Marfona (see Table 2.).

This project has identified the distribution of the principal free living nematodes affecting potato production within the UK. The distribution of the nematodes is largely static, though population levels of all the free living nematodes within sites rise and fall, sometimes quite significantly, due to a number of environmental and cropping factors. There is undoubtedly some soil and nematode transfer on farm equipment which will increase the distribution of these nematodes, but this may be limited as the soil conditions in the recipent field, with regard to sandy/silt type and also moisture requirements will often tend to mitigate against the establishment of new populations. The present distribution of the nematodes is probably largely similar to that of the past 10,000 years post the retreat of the last ice age. The virus infection present now is probably a reflection of the movement of virus susceptible potatoes systemically infected with the virus over the past 150 years or more, with the virus becoming established in small foci nematode populations and the associated field weed plant populations, with gradual increase and spread over tens of years within individual sites (Cooper *et al*.1973; Dale *et al.* 2004).

With regard to the relative importance of Tricodoid/Paratrichodorid nematodes, Lane *et al* (2000) in an overview of potato pest and disease problems described these vectors of tobacco rattle virus as medium. Ratings assigned to principal potato pests and diseases are presented in the Table 6 below (from Lane, 2000).

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TABLE 6 RATINGS OF A NUMBER OF A NUMBER OF PESTS AND DISEASES OF POTATO, FROM LANE, 2000.

Key pest, disease and other problems in potatoes requiring the use of pesticides, and their relative importance to the crop and pesticide residues (*** = high; ** = medium; * = low; – = no importance because associated pesticides not found or not sought)

Problem	**Species name**	**Importance**		**Description**
		Crop	**Residues**	
Soil pests				
Potato Cyst Nematodes (PCN)	*Globodera rostochiensis* and *Globodera pallida*	***	**	Soil-borne pests which stunt plant growth and tuber development and can seriously reduce yield
Spraing vector nematodes	*Trichodorus* spp. and *Paratrichodorus* spp.	**	**	Soil-dwelling nematodes that transmit viruses which result in internal tuber staining (spraing)
Wireworms	*Agriotes* spp.	**	–	Larvae of click beetles which tunnel into potatoes affecting quality
Slugs	*Arion hortensis, Milax gigantes* and *Tandonia budapestensis*	**	–	Slugs cause irregular shaped holes on the tuber surface extending into large cavities in the tuber affecting quality
Foliar pests				
Aphids	*Myzus persicae, Macrosiphon euphorbiae, Aulacorthum solani* and *Aphis nasturtii*	**	–	Aphids cause yield losses as a result of feeding on the foliage and also by the transmission of severe virus diseases – Potato Leaf Roll Virus and Potato Virus Y
Cutworms	*Agrostis segetum*	**	–	Cutworms cause damage by gnawing roots or emerging shoots and also make holes in tubers
Foliar diseases				
Late blight	*Phytophthora infestans*	***	*	Blight can destroy the haulm extremely rapidly, leading to reduced photosynthetic area and consequent yield reduction. Blight can also infect the tubers, leading to breakdown in store as a result of secondary infection with soft rotting bacteria.
Stem canker	*Rhizoctonia solani*	**	–	In severe cases, stem canker can completely girdle the stems and cause ‘pruning’ which leads to death of the shoots resulting in delayed emergence, gappy and uneven plant stands.

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The direct costs to the industry, given that there are no accurate records available regarding the areas affected, are difficult to estimate.

TABLE 7 ESTIMATES REGARDING THE COSTS PER HECTARE ASSOCIATED WITH CHEMICAL APPLICATION:

Product by name	Rate g/100m.drill	Rate kg/ha	Cost £/ha
Temik	86	9.5	80.0
Temik	128	14.2	116.0
Vydate	140	15.5	80.0
Vydate	210	23.0	121.0
Nemathorin	150	17.0	150.0

Data regarding the number of hectares treated in each year, or areas treated, is not available. While the survey and returns from the principal agencies highlighted the most affected areas in the UK and allowed the distribution of the nematodes and the virus to be plotted, they did not give sufficient detail to allow an estimate of the total areas affected. Discussions with the chemical companies revealed that differentiating the chemicals used for PCN control from chemicals used to control free living nematodes (see table above), often at a lower rate to control, is difficult and clear statistics are not available coupled with a degree of commercial confidentiality, resulting in estimates of up to 20,000 hectares (+/- 4,000) being treated.

Direct costs to the potato industry

Working with such estimates of 20,000 hectares and the higher rates costs of Vydate / Temik application (c. £120/ha in Table 7) to prevent spraing symptom and PCN being applied to 50% of that area and the lower rate (£80.00/ha) to the other 50% to control spraing/FLN only, this would give an annual cost of £1.2 million plus £800,000, giving a total of £2 million pound for nematicide application alone.

Data on further losses of crop within that 20,000 hectares, when nematicide provides insufficient control is not available, but if a figure as low as 1.5% on crop rejection is applied to just three spraing susceptible cultivars, with areas based on 2005 figures for England production (BPC web site) for P.Dell – c.4,600 ha, R. Burbank – 2800ha and Carlingford – 500 ha and assumptions of £110 / tonne and 40 tonnes per ha, then crop losses may be estimated at £178,000 per annum, though the figures on which these losses are based are somewhat speculative. Coupled with nematicide costs, an estimated total of £2.2 million pounds per annum to the industry would be a conservative figure.

The use of aldicarb to control FLN was estimated by the industry within the "Information Report on Essential Uses. Dec. 2004" as:

2001/02: 10210 kg 2002/03: 10383 kg 2003/04: 10633 kg
With a nematicide industry estimate of approximately 12% of the potato crop at risk, giving c. 20,000 to 24000 ha. in the UK.

Also it should be noted that the area in the UK unreported, not treated or not yet detected is unknown.

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It is clear that with few changes, the testing agencies and also the chemical companies can collect important information with regard to free living nematode distribution and total area affected within the UK ands well as for Tobacco Rattle Virus. This would require more details to be recorded than at present and also a degree of grower / company confidentiality would need to be assured.

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Lane *et al*., (2000) in the BCPC Pest and Disease Management Handbook.

Nematode control

There are no known resistance sources reported in potatoes to free-living nematodes. We are not aware of any plant resistances to FLN's being reported in the literature. As FLNs are migratory ectoparasites and remain outside the root mass and feeding from outside – with rapid probing movements – it would appear unlikely that inherent plant resistance will be identified or effective. The control of free living nematodes in the UK is largely focused on utilising nematicides to prevent the nematodes feeding, principally trichodorid nematodes, in order to prevent the nematodes transferring tobacco rattle virus and hence resulting in crop loss through levels of spraing symptoms (in spraing susceptible varieties). There is a DEFRA / LINK project LK0955 running from 2004 to 2007 which is examining the efficacy of nematicide application, modes of application and the control of PCN as well as on free living nematodes. However, while the nematicides used generally allow a crop to grow through the most susceptible period at tuber initiation time by preventing or reducing nematode movement near the root area, these chemicals do not eliminate the nematodes. Indeed the population numbers may be slightly decreased at the end of the season, possibly due to starvation or reduced vigour, but will soon recover. The nematicides applied are therefore more accurately acting as nematistats.

Alternative nematode control methods have been investigated, with efforts in this area principally in the US. Researchers there have observed that brassicas (e.g., rapeseed, mustard) have a nematode-supressive effect that benefits the following crop in a rotation. This "mustard effect" is attributed to glucosinolate compounds contained in brassica residues. Toxicity is attributed to enzymatically induced breakdown products of glucosinolates, a large class of compounds known as isothiocyanates and nitriles that suppress nematodes by interfering with their reproductive cycle. These glucosinolate breakdown products are similar to the chemical fumigant VAPAM® (metam sodium), which degrades in soil to methyl isothiocyanate. Glucosinolate compounds are also responsible for the pungent flavors and odors of mustards and horseradish. (Brown and Morra, 1997). Brown, a plant breeder specializing in brassicas at the University of Idaho,

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has released two biofumigant varieties, "Humus" rapeseed and "IdaGold" mustard, each containing elevated levels of glucosinolates. Cover crop seed for mustards, rapeseed, and oilseed radish are available from a variety of sources in the US. Oil radish as a green manure has dramatically reduced stubby root nematode (*Trichodorus*) and root lesion nematode (*Pratylenchus*) in Idaho potato fields. (Anon, 2001). Rapeseed and sudangrass green manures grown prior to potatoes at Prosser, Washington, provided between 72 and 86 percent control of the root-knot nematode in that crop. (Stark, 1995) However, in the same study, on-farm research in western Idaho showed that rapeseed green manures decreased soil populations of rootlesion nematodes to a greater extent than did sudangrass green manures. Winter rapeseed and canola should be incorporated in very early spring. (Cardwell and Ingham, 1996). For root lesion nematode control on potatoes, researchers in the US found that forage pearl millet (Canadian Hybrid 101) and marigold (Crakerjack) as rotation crops with potatoes resulted in fewer root lesion nematodes and increased potato yields than rotation with rye. (Ball-Coelho *et al.,* 2003).

Marigold (*Tagetes species*) is one of the most highly studied crops for its ability to suppress nematodes with antagonistic phytochemical exudates, namely the polythienyls. Research also demonstrates that rhizobacteria living in association with marigold roots are suppressive to root lesion and other nematodes. These multiple effect nematode-control properties can benefit other crops when marigolds are grown in rotation. (Sturz and Kimpinski, 2004) African marigold (*Tagetes erecta*) and French marigold (*Tagetes patula*) are popular ornamentals in the horticultural trade with several nematode-suppressive varieties each. (Dover *et al.,* 2003).

While there are other approaches such as flooding or searching for other biocontrol agents such as fungi with known nematophagous activity, however, these are based on *in vitro* laboratory experiments which are difficult to extrapolate to field scale and practices. The above brief summary of research on plant residues affecting free-living nematode populations may offer possibilities with regard to reducing or managing free-living nematodes if they can find appropriate place within crop rotations. There is a paucity of research in the UK with regard to this subject area.

With the projected withdrawal of Temik as a nematicide to control free-living nematode damage to crops it may be timely to initiate a limited assessment of the potential of some plant species to control populations within the UK environment.

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TRV resistance as a target for selection and breeding

Resistance to infection by Tobacco Rattle Virus is known to exist, though as yet it has not been accurately identified. Research at SCRI investigating the reaction to TRV infection (cause of ‘spraing’ or corky ring spot symptoms in tubers) of a population of potato genotypes derived from the hybridisation of a 'susceptible' x 'resistant' cultivar indicates that inheritance in this population is simply inherited. Resistance to TRV in potato cv. Record appeared to be determined by a single resistance gene. AFLP markers linked to this putative gene have been identified by bulk segregant analysis. The results of the population studies also indicate that the resistance to TRV infection is separate from the heritable system responsible for the ‘spraing’ response in potato tubers. The marker demonstrates the potential of MAS in the case of disease resistance that at present relies on field trials over a number of years for a virus that is often distributed in patches associated with the distribution of its nematode vectors within sites, making assessment difficult and sometimes unreliable. The AFLP band associated with TRV resistance in segregating population is arrowed in Figure 11.

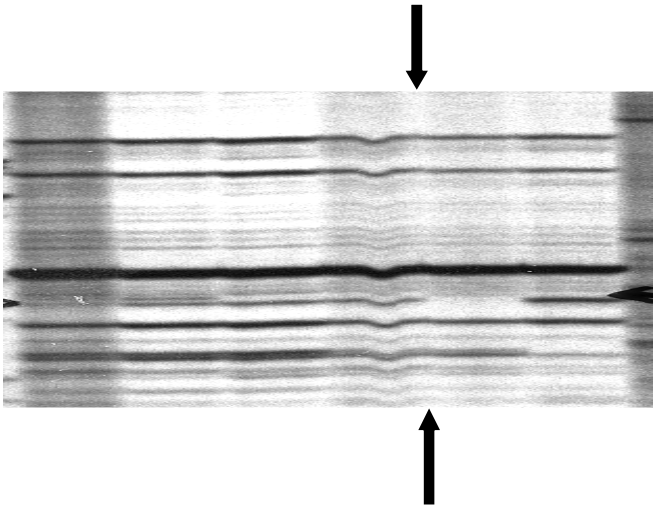

FIGURE 11 AFLP BAND ASSOCIATED WITH TRV RESISTANCE

However, the marker is not diagnostic of resistance in a wider range of germplasm and hence further research is required to locate the gene more accurately.

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Useful source of information

http://www.inra.fr/Internet/Produits/HYPPZ/RAVAGEUR/6prapen.htm
www.bayercropscience.co.**uk**/pdfs/tactics_to**_control_spraing_**96.pdf
http://www.scri.sari.ac.uk/programme1/TRV-resistanceepidemiologyanddetection.htm
http://www.csl.gov.uk/prodserv/diag/potato/Soil_testing_spraing.cfm

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Recommendations

1. There is a need for agencies that test for free living nematodes and for the presence of Tobacco Rattle Virus to standardise the data they collect to contribute to a coherent body of data that will give informative statistics on the distribution and also the area of land affected. Data to include: standard Ordinance Survey grid reference points would appear appropriate. Also number of tests and total area assessed to be positive and/or negative. This could be facilitated through a workshop forum of appropriate testing bodies and advisors and could be in place within a period of a few months. Data collected over a 6 or 7 year period would represent a comprehensive body of data not currently available. It is important to include the principal nematicide producing/ distributing companies within this forum.

2. There are now a number of diagnostics for free living nematodes and the presence or absence of TRV. It is important that these newly developed tests are properly validated, compared and the reliability of the tests ascertained and information given to the growers. A project would require the identification of a number of appropriate potato production fields in England (ware) and Scotland (both ware and seed) and the assessment of risk involved in the year prior to the potato crop, followed by observed levels of spraing symptoms in the spraing-susceptible potato crop. From the initial results looking at limited fields and test procedures (BPC spraing diagnostic procedures using weeds as bait plants – R255 report on BPC web site) it is important that the fields include those fields with low levels of viruliferous nematodes at those sites producing 1% to 5% appear the more difficult to detect / predict. A project would require c.3 years and could be conducted – in part – on some ‘model’ farms to ensure growers are informed & included.

3. With the increasing controls on the application of nematicides to control free living nematodes and Tobacco Rattle Virus, there is a need to investigate alternative ways forward for the industry. This includes: a) the investigation of green manures (in set aside) to reduce FLN populations as already piloted in the US, b) the use of some crops to ‘clean up’ TRV vectors of the virus (alfalfa crops in US). This would require the identification of a number of sites to which different treatments would be applied. The biofumigant rapeseeds and mustards developed in the US may merit trialling e.g. “Humus” rapeseed and “IdaGold” mustard, each containing elevated levels of glucosinolates. A timescale of 3 to 4 years would probably be required.

4. A number of varieties e.g. Wilja, King Edward, Sante, Arran Consul, Nadine, Shepody, Saxon, Marfona and Rocket are known to be susceptible to the virus and become systemically infected with the virus and remain so through many years (in excess of 6 years). Such varieties represent an important mode of transfer of the virus over the past decades of movement of potatoes (seed transferred between different countries and different farms and home-saved seed within farms), thus transferring the virus from site to site. However there is a requirement that we correctly classify potato varieties grown in the UK / Europe to identify other susceptible varieties that may act as carriers of the virus. This would require growing plots of targeted varieties (e.g. those with a NIAB rating of 6 to 8) in TRV infected soil and testing the seed produced over c. 2 year period for persistence of

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the virus and for systemic virus infection (as in Dale M F B *et al*. 2004**.** Effects of systemic infections with Tobacco rattle virus on agronomic and quality traits of a range of potato cultivars. *Plant Pathology* **53**:788-793.). The work would not require dedicated labour / technical support throughout the project.

5. The longer term strategic approach to controlling virus symptoms / spread and the reduction of chemical usage within a sustainable system must lie in part with the development of resistant varieties. The identification of true inherent resistance to the virus and also of separate significant heritable factors responsible for the development of spraing symptoms offer real opportunities for the development of such varieties. Breeders currently lack robust testing procedures to assess breeding material and select improved varieties, relying on field trials over a number of seasons to test restricted numbers. Significant progress could be achieved through the development of robust molecular markers closely linked to identified virus resistance to aid/assist longer term breeding efforts to produce TRV resistant varieties. Project would take approximately 4 years and would be cost-effectively completed within a PhD training programme for a 3 year student. Material in the form of robust molecular markers for application within breeding programmes and proven parental material would lead through to resistant varieties.

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Appendix 1. Spraing Advisory Notes for Growers

Effects of Tobacco Rattle Virus on potatoes

Introduction

It was previously thought that Tobacco Rattle Virus (TRV) was self-eliminating from seed potato stocks and that the main effects of this nematode-transmitted virus were the visible spraing symptoms of arcs or lines of necrotic corky tissue formed within the tuber flesh. However, research funded by SEERAD/BPC, has clearly demonstrated that the virus can become established systemically in a number of widely grown potato cultivars which exhibit few, if any, tuber flesh symptoms. Such systemically infected cultivars can carry the virus, over a number of tuber 'generations', from year to year i.e. that the virus is not self-eliminating. These cultivars can act as a reservoir for 'clean' or non-viruliferous nematodes to pick up the virus during feeding. Hence, such cultivars are an important mode of transmission of this virus between sites/fields.

The serological methods used for diagnosis of most potato virus infections are inappropriate for TRV. RNA-based methods (RT-PCR) that are effective and reliable have been developed and are now available.

Following assessment of a range of cultivars, most appear to fall into one of three groups:

1. *Resistant*: those varieties that do not show any symptoms and from which we cannot detect or recover any virus. Usually in NIAB rating 7 to 9.
2. *Spraing sensitive:* cultivars, which exhibit classic 'spraing' symptoms of arcs/lines/spots of necrotic corky tissue. Virus particles are rarely found. Is a 'self-eliminating' infection. Nematodes, when feeding on roots of plants, do not acquire the virus. Usually in NIAB rating 1 to 3.
3. *TRV susceptible:* those cultivars that show few, if any, symptoms in the tuber flesh, but can become systemically infected. Is <u>not</u> self-eliminating. Infected plants can act as a reservoir of virus by carrying the virus from site to site with 'clean' nematodes at a new site able to acquire the virus on feeding. Cultivars in this group tend to have a NIAB rating of 5 to 7.

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Initial grouping of some UK cultivars:

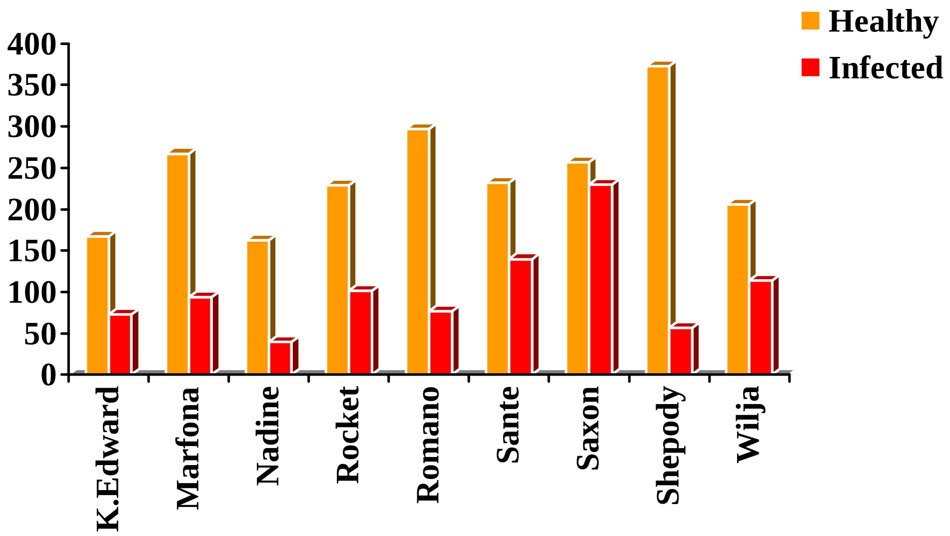
Group1: **Resistant**		Group 2: **Spraing sensitive**		Group 3: **TRV Susceptible**	
Arran Pilot	(9)	Pentland Dell	(1)	Wilja	(5)
Bintje	(7)	Maris Bard	(2)	King Edward	(6)
Record	(9)	Picasso	(1)	Santé	(6)
Saturna	(7)	Russet Burbank	(1)	Arran Consul	(6)
Climax				Nadine	(6)
Nicola	(8)			Shepody	(6)
Lady Rosetta	(8)			Saxon	(7)
Fianna	(80			Marfona	(6)
				Rocket	(5)
				Home Guard	(7)

It is now evident that such virus infection can have a dramatic and detrimental effect on a number of important agronomic and quality characters, and the results are summarised below. As a group, the cultivars that can carry the virus systemically while exhibiting few if any sparing symptoms account for approximately 30-35% of the potato area in the UK.

Size

From the chart below it is evident that when fully infected systemically, plants tend to produce significantly more tubers, but that these are noticeably smaller, rendering a greater proportion of the produce unsaleable.

Effect of TRV on ave tuber weight (ave. tuber wt. Grams)



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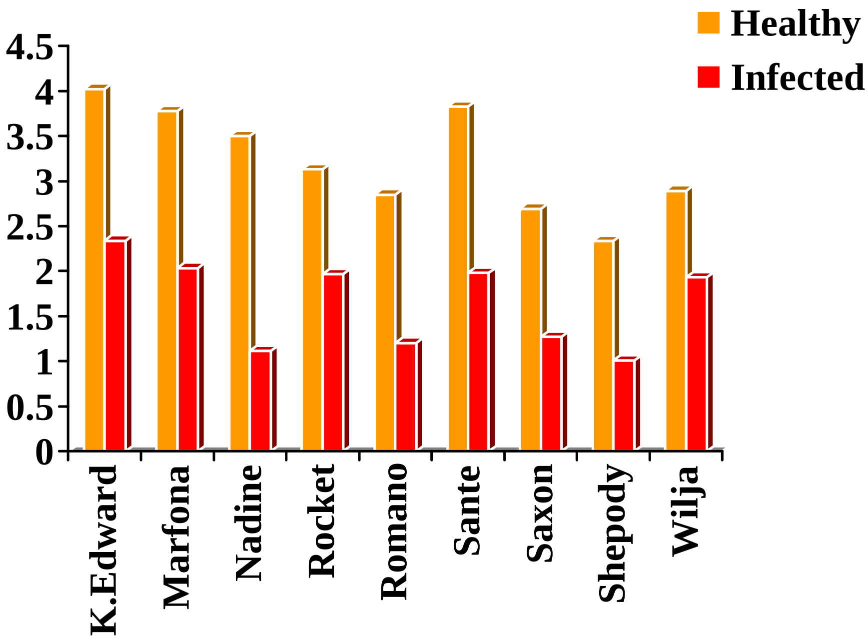
Yield

The virus also has significant effects on the yield of these cultivars; generally lowering overall yields as can be seen in the yield charts . The tubers produced on infected plants are often misshapen by secondary growth and/or growth cracking (see photographs on page5).

TRV - Effect on yield
Ave. wt / plant kg



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Quality

After-cooking blackening (ACB) is often noticeably more severe in infected material. The levels of the principal cause of ACB, chlorogenic acid, are also noticeably higher.

3.TRV - Effect on quality in *cv.*Wilja

	Healthy	TRV infected
Chlorogenic acid *mg/100 g Freeze Dried Matter*	78.41	116.16
After Cooking Blackening *1 = poor, 9 = good*	5.75	4.31

From the results it can be seen that the virus can markedly affect the growth of plants of certain cultivars when fully systemic. Effects will probably not be obvious in that infected plants will be distributed at random in a crop exhibiting limited, if any, foliage symptoms. These results have implications for how the virus is transmitted from field to field, region to region and even from country to country.

Examples below demonstrate how TRV affects the growth characteristics in a wider range of cultivars to different degrees including emergence, tuber malformation and foliage symptoms.

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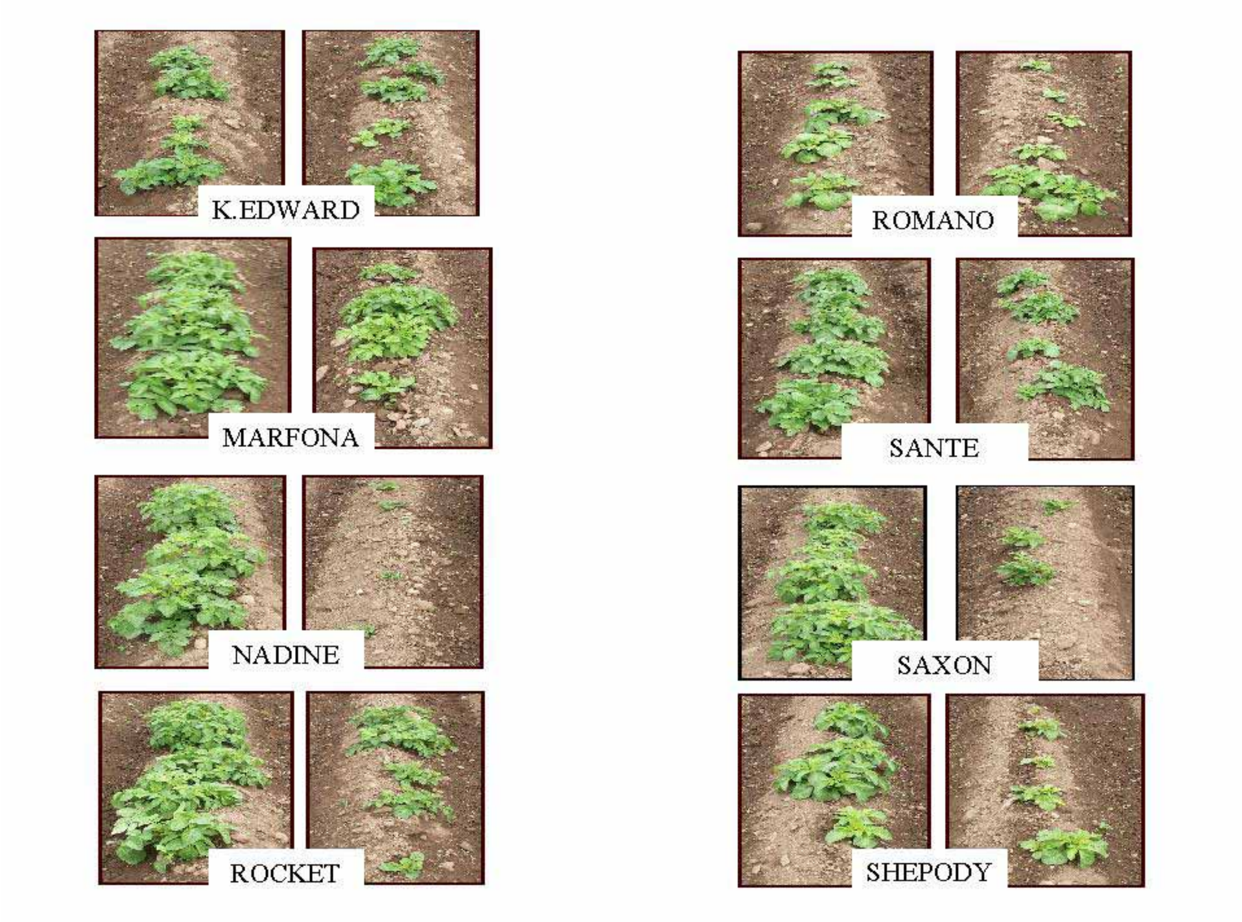
Plant Emergence / Establishment



Plot emergence: healthy (left) compared to TRV infected (right) of some common cultivars.

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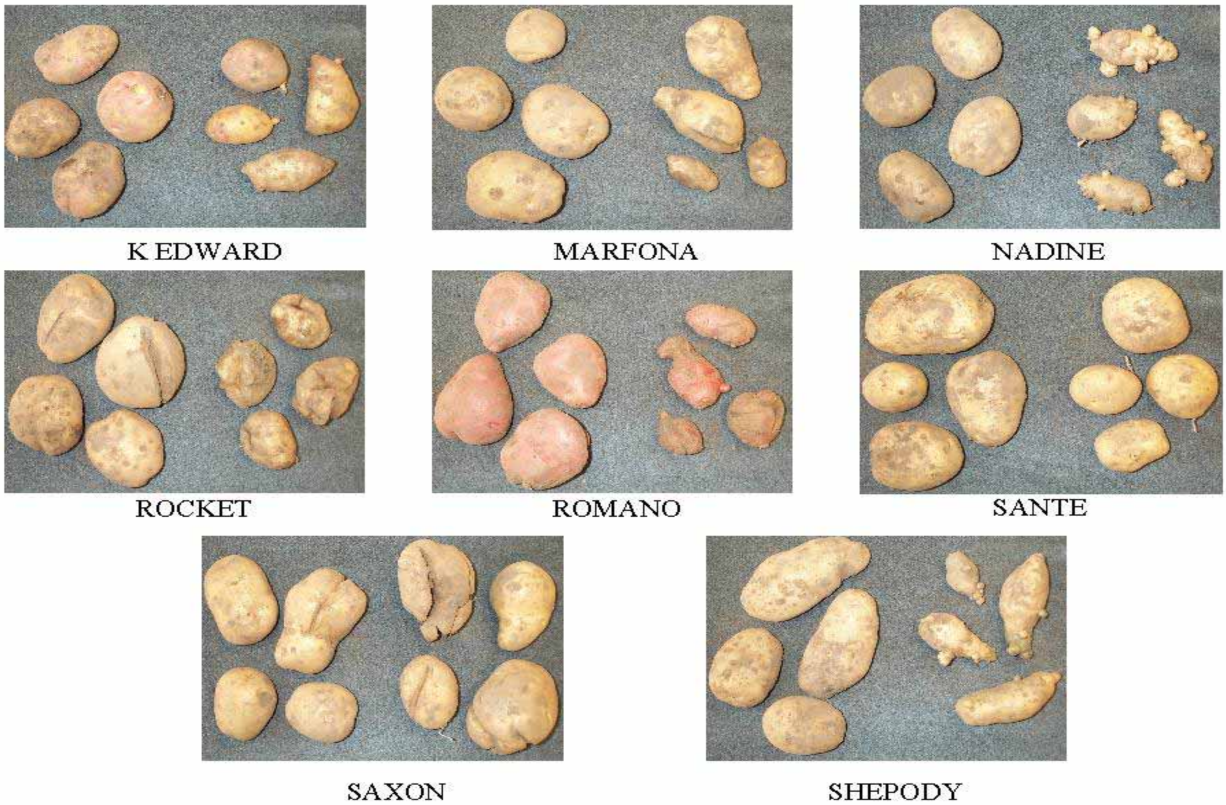
Tuber malformation



Comparison of harvested tubers of 'susceptible' cultivars: healthy tubers on left & TRV systemically infected tubers on right.

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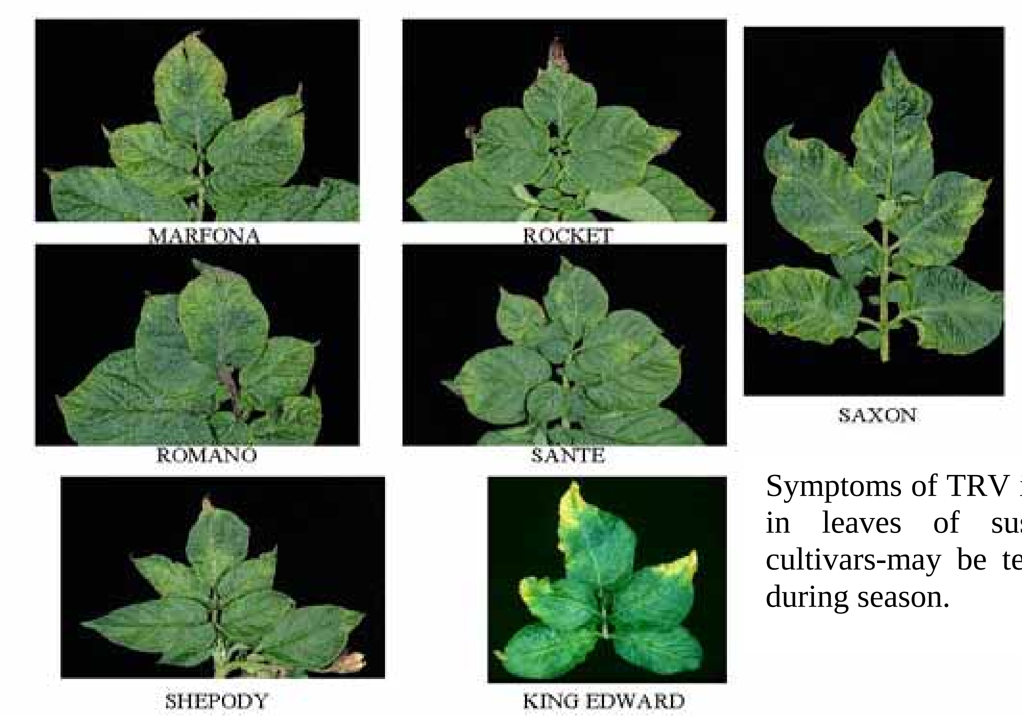
Foliage symptoms



Symptoms of TRV infection in leaves of susceptible cultivars-may be temporary during season.

Virus and weeds – persistence within sites

The virus has a very wide host range. It can infect more than 400 monocotyledonous and dicotyledonous species. The virus does not usually become systemic in most of these hosts, often remaining in the roots of plants that do not exhibit foliage symptoms, but several are invaded systemically and some of these, such as *Stellaria media*, may show no obvious symptoms. Some of the more common agricultural weed species known to carry the virus are given in Table 1 and such weeds act to perpetuate the virus within sites and nematode populations. In a few cases the virus can be transmitted through the seed, with up to 10% transmission rates found in *Viola arvensis* (Cooper and Harrison, 1973).

Weed	**Common name**	**Weed**	**Common name**
Capsella bursa-pastoris	shepherd's purse	*Senecio vulgaris*	groundsel
Chenopodium album	fathen,lambsquarters	*Stellaria media*	chichweed
Polygonum aviculare	knotgrass,knotweed	*Taraxacum officinale*	dandelion
Polygonum convolvulus	bindweed,wild buckwheat	*Viola arvensis*	field pansey,violet

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TRV - Notes & Control Measures

1. In spraing-susceptible cultivars, infection is gradually self-eliminating and there is generally minimal risk of transfer of virus e.g. P. Dell, M. Bard.
2. In general, the milder the symptoms, the greater the level of transmission to daughter tubers/plants.
3. In virus susceptible cultivars, all/most daughter tubers are infected. These can act as sources for virus acquisition by nematodes at a new site/field.
4. For TRV to be introduced to a new site via infected seed, the appropriate nematode vector for the particular TRV strain must be present.
5. There is a poor relationship between nematode numbers and the risk of TRV infection, also large sampling error. Nematodes have a patchy distribution. Nematodes tend to be found in lighter / sandier soils.
6. There are different strains of TRV. Different strains may cause different reactions in different potato varieties.
7. Weed control is important. Hosts of TRV include common weeds such as field pansy, knotgrass, shepherd's purse and groundsel, and the virus is seed-borne in most of these. Organic practices and set-aside may facilitate the re-introduction of TRV and / or the increase the distribution of the virus within a field.
8. Continuous barley will decrease the virus (not a good host) **but** will increase the nematode numbers - therefore **<u>weed control must be good</u>**. May be possible to eradicate virus over (guesstimate) a 5 to 7 year period this way. Also, alfalfa reported in US as reducing virus levels over 2-year period - must have **no** weeds.
9. Use of chemicals to control TRV - immobilises rather than kills nematodes, allows crop to be grown. The nematodes and virus are still present after the crop. Soil sterilisation e.g. telone will kill nematodes - but will eventually reappear from further down the soil profile.
10. Do not use home-saved seed from crops which showed spraing symptoms or of varieties in the ‘susceptible’ class from spraing affected soils.
11. Moisture is required by nematodes - e.g. in wetter areas/irrigation. Nematodes will move up or down the soil profile in response to soil moisture. Nematodes are susceptible to desiccation.
12. Length of rotation unimportant.
13. Field history, particularly recent, regarding TRV/spraing is useful information. Set aside containing many weeds may increase the virus if present.
14. Some plants can reduce nematode numbers e.g. asparagus, (horseradish).
15. Choose cultivar/seed source with care e.g. seed tubers of Nadine/Wilja not from TRV-affected or sandy soils.
16. Once soil has TRV, it is very difficult, if not impossible, to eradicate.

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Pre-Plant Detection of TRV and Trichodorid Nematodes

Trichodorid nematodes (*Paratrichodorus* and *Trichodorus* spp.) are the natural vectors of TRV. These nematodes are small, 0.6 to 1.5 mm long, ectoparasites that live in the soil and do not enter plant tissue. They have extensive host ranges, and live for two to four years in the soil. The adult, having acquired TRV, probably remains viruliferous for the remainder of its life.

TRV has a world-wide distribution, being particularly prevalent in Europe, the western regions of the former Soviet Union and associated countries, and North America. The principal crop damage is 'TRV-spraing' disease in potatoes in Europe and North America and in high value bulbous flower crops in the Netherlands. Pre-planting detection of the virus is traditionally done by 'bait-testing' soil samples, i.e., growing herbaceous plants in soil and subsequently testing the roots for the presence of the virus. This method takes approximately six to eight weeks, requires the service of a trained plant virologist, or if using Taqman, may be reduced to 4 to 5 weeks.

There is a specific association between serologically distinguishable strains of TRV and individual species of *Paratrichodorus* and *Trichodorus* nematodes. In the UK, *P. pachydermus* and *T. primitivus* and the TRV serotypes PRN and RQ, respectively, account for approximately 80% of trichodorid spp. and TRV strains encountered in samples collected from potato sites. In the Yorkshire area, *P. anemones* and its associated strain of TRV are most common, and other trichodorids only infrequently encountered in association with strains of TRV are *T. cylindricus*, *T. similis* and *T. viruliferus*. The results from recent soil bait testing of a limited number of samples received from potato areas in Scotland, and from Yorkshire, East and West Midlands, and the Southampton areas of England, revealed that approximately 50-60% of the samples contained trichodorids. Five to 20%, depending on the area, of these samples contained trichodorids in association with TRV. Approximately 25% to 35% of targeted sandy & silt soil samples examined in the Lothian, Borders, Fife, Perthshire/Angus and Tayside areas in Scotland appeared positive for the virus.

Currently, pre-planting risk assessment for TRV and its associated vectors is based on screening soil samples for the presence of trichodorid nematodes and collecting site history data. Recommendation to apply chemical to temporarily suppress the activity of the nematodes, hence preventing the nematode from transmitting virus, is based principally on the number of trichodorids present in a sample, and not on direct detection of TRV in a sample. It is recognised that the occurrence and transmission of TRV by trichodorids is not related to the size of the nematode population.

Recently developed PCR tests will show if you have nematodes, and if they are carrying TRV. Nematodes move freely through the soil, so need to sample soils in warm, moist conditions, to a depth of at least 20cm. Sample soils usually in late autumn or early spring. Handle soil samples with care to avoid killing nematodes.

Risk assessment of virus risk is a necessary part of product justification within an Integrated Pest Management strategy. Growers may need to apply nematicide where they have a risk of crop rejection due to spraing symptoms and also review their choice of variety if possible.

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Agency laboratories undertaking pre-crop soil tests for FLN and Tobacco Rattle Virus presence include:

SAC in Edinburgh: your SAC adviser is in your local directory
SCRI in Dundee: 01382 562731
ADAS High Mowthorpe: 01944 738646
NIAB Cambridge: 01223 276381
CSL Sand Hutton: 01904 462000

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