Project Report

Improvement of a diagnostic test to allow more precise localisation of tobacco rattle virus in fields

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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.

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. Current practice is to treat the whole field with nematicide should any TRV be identified, but a more targeted application might be possible if the distribution of the virus were accurately known. It is therefore important to know the fields and parts of fields where TRV occurs, and where control measures may be required. Because nematodes and virus spread only gradually, mainly through soil movement (e.g. ploughing) or in the seed of a few weed species, knowledge of the distribution of TRV is likely to remain applicable for several years.

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 are 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.

Aims of the project

The rationale behind the present project was that weeds are in effect *in situ* bait plants that could be used as indicators of the presence of the virus. Work was therefore undertaken to compare the detection of TRV, using TaqMan technology, in the roots of weeds and of glasshouse-grown bait plants. Occurrence of virus would be mapped in detail within 2 ha sites using TaqMan molecular procedures both on the roots of weeds and (where applicable) of crop plants e.g. barley, and on conventional bait plants. In addition, the distribution of virus found in these tests was compared with the occurrence of spraing symptoms in a potato crop in the following year.

Summary of work undertaken during the project and key findings

Three TRV infected sites were identified in collaboration with industry partners. All three sites were growing cereal crops in 2004, which allowed easy weed sampling, and were planned for potato production in 2005.

Site 1: Near Branston in the east Midlands, England. Light sandy loam. Field sown to winter wheat (cv. Hereward). Weeds well developed when sampled on 14 April 2004.

Site 2: Near Brechin in Angus, Scotland. Sandy loam soil. Field sown to spring barley. Weeds small at time of sampling (29 April 2004) about 4 weeks after seedbed preparation.
Site 3: at Newport, near Dundee, Scotland. Very sandy soil near sea/ estuary. Field sown to spring barley. Sampled on 11 May 2004, about 6 weeks after seedbed preparation.

At sampling, weeds and soil were taken from points on a 25m grid covering about 2 ha. The roots of the weeds were tested for TRV by TaqMan within 48 hours. Petunia bait plants were planted in the soil samples and, after about 5 to 6 weeks, their roots were also tested by TaqMan

In 2005, the identified plots within the three sites were planted with spraing-susceptible potato cvs (Pentland Dell or Pentland Javelin). At harvest, samples of tubers were taken from the same grid points as before, and scored in store for the occurrence of spraing symptoms.

Results

At site 2 (Brechin), few of the 2004 samples were positive in either the weed and the glasshouse bait tests, and there were few spraing symptoms in 2005 and those that were observed were not severe.

The results from the other two sites in 2004 confirmed that the roots of weeds contain the virus, can be used to assess its presence within fields, and can give some indication of its distribution. Maps of the distribution of virus as determined by each of the two tests, and of the distribution of spraing symptoms in 2005 are shown in Appendix 1 and Appendix 3 to this report. There was a good agreement between areas with high virus incidence and spraing symptoms, and between areas identified as virus-free and no spraing symptoms. The overall pictures were however clouded by areas in which the virus appeared to be at a low level. In such areas, the lack of a well-defined threshold in the TaqMan test made it difficult to be sure whether or not virus was indeed present, and seasonal environmental factors will have had a disproportionate influence on the occurrence of spraing.

Practical recommendations for growers

TRV was successfully identified in the weeds at each site as in the glasshouse bait plants. Thus the detection of the virus in a field by a method based on weed sampling should be at least as reliable as any of the tests currently on offer. The rapidity and simplicity of a test on weeds would allow more intensive sampling of a field and the possibility of accurately mapping the distribution of TRV.

Moreover, sampling by collecting representative weeds could be done by growers themselves at any time when there is a reasonable weed flora in the field, in contrast to soil sampling, which requires some expertise and must be done when soil conditions are suitable for nematode activity.

The potential for higher resolution mapping of the virus distribution within a field remains unproven. Both tests detected risk areas, where high levels of virus detected in 2004 were correlated with high levels of spraing in 2005. Most useful however would be the ability to identify substantial parts of fields that are virus free, with a view to leaving these areas untreated with nematicide, but neither test did this 100% reliably, largely because of the difficulty in distinguishing between very low and zero levels of virus. It should also be remembered that the level of virus is not a direct indicator of the risk of spraing, which is greatly affected by seasonal environmental factors, such as soil temperature and moisture content at critical times during crop development.

Experimental section 2004

Introduction

Infection by Tobacco Rattle Virus (TRV) causes a number of different responses in potato plants including necrotic arcing (known as spraing, corky ringspot) in the tuber flesh, and stem-mottle (distortion, stunting and mottling, typically confined to one or a few of the shoots produced from an infected tuber) and aucuba in the foliage (Harrison and Robinson, 1981). Information with regard to the UK area affected by TRV is inadequate, though industry sources familiar with nematicide applications to control the free living nematodes 'guesstimate' that some 10,000 hectares are treated each year with £900,000 worth of chemicals. Tubers exhibiting spraing symptoms can give rise to virus-free progeny plants. Systemic infections exhibiting few symptoms in the tuber also occur. The virus 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 are invaded systemically and some, such as *Stellaria media*, may show no obvious foliar symptoms. The virus host range includes many common agricultural weed species, and such weeds 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). Distribution of the virus in the soil reflects that of its vectors, which prefer light and/or sandy soils (Cooper, 1971). 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. The simplest solution is to avoid growing ware crops of spraing-sensitive cultivars on land where TRV occurs. Indeed, this is the only solution available to organic growers. Moreover, like all soil-borne viruses, TRV is nearly impossible to eradicate from a site, and it is therefore particularly important not to introduce it to new sites. In this connection, the realization that infected potato tubers can be sources for transmission of the virus is significant. Thus, it is inadvisable to use TRV-affected land for seed production, even of cultivars that are not affected by spraing. A further pressure on growers is the demand by supermarkets and the supply chain that the use of nematicides for spraing control is justified on a reliable basis.

It is therefore important to have a detailed knowledge of the fields and parts of fields where TRV occurs. The distribution of TRV within a field is often patchy, and the virus spreads only gradually, via soil movement (e.g. ploughing) and limited true seed dispersal. Therefore, such 'mapping' of infections would be durable probably over a number of years, say 5 to 10 years, and would allow a grower/adviser to target risk areas for nematicide application or crop avoidance. Existing methods of predicting the occurrence of TRV rely either on counts of trichodorid numbers, which do not necessarily correlate with the presence of virus, or on various bait test systems. Bait tests are reasonably reliable, but take more than a month to complete and require large amounts of glasshouse space and are a significant cost to growers. Therefore, the number of samples taken per field is usually quite small, leading to poor definition of affected areas, if indeed they are successfully identified. Consequently, current practice is to treat the whole field with nematicide should any TRV be identified. A development of the bait test is now being offered through the Central Science Laboratory which involves collecting soil samples, using bait plants and after 3-4 weeks using real time

quantitative polymerase chain reaction (TaqMan) technology to assess the presence/absence of TRV in the roots of the bait plants. A further diagnostic is the use of improved nematode extraction systems and TaqMan testing for viruliferous nematodes as developed at SCRI (BPC/SCRI).

Initial Experiment And Results

As described earlier, TRV is primarily found in weed species and, importantly, this is how it is maintained within a site and its nematode population. Earlier published research has identified some of the most common weed hosts. In preliminary work during 2003 at SCRI, using a site where TRV was known to occur, the roots of weeds within a developing barley crop about 4 weeks after emergence were tested for virus infection, with the following results:

		No. +ve/total no. tested
Chickweed	(Stellaria media)	10/11
Fathen	(Chenopodium album)	3/14
Mayweed	(Matricaria discoidea)	2/ 3
Groundsel	(Senecio vulgaris)	1/ 3
Small nettle	(Urtica urens)	0/ 6
Hemp nettle	(Galleopsis sp.)	0/ 3

The numbers and species tested merely represent the weed flora at that particular site, but it is clear that some species are more susceptible than others. We also examined the roots of some of the barley plants, and detected TRV infection by RT-PCR in the roots of about 2-3% of them. These findings demonstrate the feasibility of developing a rapid (1-2 days) assay based on established reliable TaqMan molecular procedures for the presence of TRV in field situations. The simplicity of such a test would allow more intensive sampling and thus the accurate mapping of a field for the presence/absence of TRV and the risk of crop infection. It also offers a test that can potentially be applied at almost any point in a rotation rather than during a short 'window' prior to planting potatoes and will improve disease management. This short project examined the applications of such a test.

Materials and Methods

The project studied known TRV-affected areas within three fields prior to potato cropping. The objective of the project was to study the occurrence of virus in the fields and attempt to map, in detail, the 2 ha sites using TaqMan molecular procedures both on the roots of weeds within the sites and (where applicable) the roots of crop plants e.g. barley. The project also applied the conventional bait soil/testing as currently used within the industry. In the subsequent year (2005) the produce of spraing susceptible potato crops were sampled and the predictive ability of the diagnostics assessed. The results of these field-tests undertaken within the project allowed direct comparison of the tests and the ability to accurately locate and delimit areas with viruliferous nematodes.

Three sites were identified as known to have some TRV viruliferous nematodes present and were fields planned by the growers to be planted to potatoes in 2005. The project had planned to sample the weeds and soil within winter grown cereal crops, during the early spring where possible, in fields identified by industrial partners. The following three sites were identified:

- Site 1: near Branston in the east Midlands, England
  Field sown to winter wheat (cv. Hereward) – light sandy loam – sampled on 14 April 2004, weeds well developed.

- Site 2: near Brechin in Angus, Scotland
  Field sown to spring barley, sampled 29/04/2004 c. 4 weeks after soil / seedbed preparation – sandy loam soil. Weeds small at time of sampling.

- Site 3: at Newport, near Dundee in Scotland
  Very sandy soil near sea/estuary – the site is well known to SCRI staff. Sampled on 11 May 2004 c. 6 weeks after soil/seedbed preparation.

Weeds and c. 2.0 kg soil were taken from points in a grid system c. 25 metres apart covering c. 2 ha, depending on local field situation. The areas are mapped according to these tests in attached appendices. The roots of weeds were prepared within 48 hours of field sampling. Indicator bait plants – petunia – were planted in soil samples brought from each of the individual sampling points within the sites to allow comparison between the weed assay and the conventional bait test system. The bait plants were allowed to grow and after c. 5 to 6 weeks were tested for presence of virus, as were the weeds brought directly from the fields. Testing for the presence of TRV was as described below, based on the method of Mumford et al. (2000).

Details of sample number and extraction procedure at the three sites during 2004

Site	Sample		RNA extraction method
1 (Branston)	Weed roots	45 samples	[1]Qiagen
1	Bait petunia roots	45 samples	[2]Kingfisher
2 (Brechin)	Weed roots	51 samples	Qiagen
2	Bait petunia roots	51 samples	Kingfisher
2	More weeds	25 samples	Kingfisher
3 (Tayport)	Weed roots	44 samples	Kingfisher
3	Bait petunia roots	44 samples	Qiagen
3	Barley roots	44 samples	Qiagen
tests	Clean petunias	14 samples	Kingfisher

[1]Rneasy plant mini kit (Qiagen)
[2]Total RNA purification kit for Kingfisher mL (Thermo Labsystems)

Sample preparation 2004

Weed roots were washed and samples taken from at least four separate plants. As chickweed (Stellaria media) is known to be a good host, it was included where possible. At site 2 the weeds were so young and small the size of chickweed was noted in case very young plants were not infected.

Petunias were grown in soil sampled from the field on the same day the weeds were collected. They were grown for around 5 to 6 weeks then washed out and bagged and stored at 4°C. Again a sample was taken from each of four plants. Fresh gloves were used for each sample to minimize cross contamination.

RNA extractions

100mg washed roots (sampled from four plants) were ground in liquid nitrogen, in a 1.5ml tube with a plastic pestle, for 1 min then lysis buffer added (0.450ml Qiagen; 0.900ml Kingfisher mL) and further grinding until thawed and well mixed. Samples placed on ice immediately. Fifteen samples were processed at one time. After grinding, a tungsten carbide bead (3-mm Qiagen) was added to each tube and shaken on ball mill for 90 secs at 30/sec. Samples were then spun at 3000 rpm for 1 min and either [1](Qiagen) loaded onto shredder column or [2](Total RNA purification kit for Kingfisher mL) loaded into tube strip with 50 µl magnetic beads. Protocol was then followed as per instructions for appropriate kit.

Qiagen extractions were suspended in 45 µl, kingfisher extractions were eluted in 200 µl.

PCR TaqMan

Samples were run in duplicate and positive controls were included as well as no template controls.
Where Qiagen extractions were used the template was 3 µl. A 5 µl template was used with the Kingfisher extractions.

12.5 µl	Mastermix
1.0 µl	F primer (7.5 µM)
1.0 µl	R primer (7.5 µM)
1.0 µl	probe (FAM) (3.75 µM)
0.125 µl	MuLV RT (200 U/µl)
3.0 µl	template RNA (5 µl template RNA Kingfisher mL)
6.375 µl	SDW (4.375 µl SDW Kingfisher mL)

Make MASTERMIX first
ABI prism plates + optical caps
Samples in plate were spun for 1 min at 300RCM and lids checked with plastic tool.

Thermal cycler conditions

48° 30 MIN
95° 10 MIN
60° 1 MIN
95° 15 SECS
} X40 CYCLES

25 µl sample

Results were downloaded after amplification plots were checked and baselines altered if necessary.

Routine Cleaning

Tungsten carbide beads were cleaned for 1 min in 0.4 M HCl and rinsed in SDW.
Plastic pestles were washed and then soaked 1 hour in 0.1 MNaOH 1m MEDTA and rinsed in DEPC water then autoclaved.

Tests

RNA was extracted from clean petunias, interspersed with samples known to be infected with TRV, to test for cross contamination between samples. We also wanted to determine a "cut off" for TRV positive.

Dnase treatment

Samples which were used to test for crossover contamination (see tests in table above) were Dnase treated (Ambion DNA-free) and run on Taqman to check for amplification from DNA in samples.

RT-PCR from root exudates applied directly to FTA Cards (Whatman)

Approximately 400 mg root was ground and exudates collected in a 1.5 ml tube. 25 µl of this sample was applied immediately to the centre of a FTA card (Whatman). Samples were taken from roots, samples of which had already been tested by Taqman for presence of TRV. The machine rollers were cleaned between samples by washing with 0.2M NaOH followed by 96% ethanol and 10 secs of water jet washing. Samples were left to air dry for 1 hour before packing into desiccating pouch. A positive control was included where 25µl total RNA was directly added to the paper. A negative control where Rnase free water was added was also included.

2 mm cores were removed from each filter by corer (cleaned between each sample by ethanol and coring a clean filter) and placed into a 1.5 ml tube. 400 ml of RNA processing buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 800 U.Ml Rnase Out (Invitrogen), 200 µg/ml glycogen, 2 mM DTT) was added to each tube mixed by pipette and incubated on ice for 15 minutes with mixing by vortex every 5 minutes. The RNA has now been removed from the disk so the paper was removed from the tube. RNA was then precipitated by $1/10^{th}$ volume 3M sodium acetate and an equal volume of ice-cold isopropanol. Each sample was kept for 1 hour at –20°C and then centrifuged at maximum speed for 15 minutes, supernatant removed and pellet washed in ice cold 75% ethanol and spun for 5 minutes at maximum speed (12,000xg). The supernatant was removed and the pellet air dried for 15 minutes then RNA resuspended in 20 µl water (Rnase free) heated to 58°C for 5 min and then stored frozen at -20°C.

RT-PCR was carried out as before using 2 µl as template including previously extracted RNA as a control.

Results

The principal weed species at the three sites are listed in Tables 1, 2 and 3 for sites 1, 2 and 3 respectively. At site 1 *Senecio vulgaris*, groundsel was predominant, with 13 samples containing *Stellaria media* (chickweed). At site 2 *Stellaria media* (chickweed) predominated, being present in all samples. At site 3 *Stellaria media* (chickweed), *Senecio vulgaris*, groundsel and *Chenopodium album* (fathen) were the principal weeds sampled, all known as hosts for the virus.

The results obtained for the weed root assay and for the soil bait tests are presented for the three sites in Tables 4, 5 and 6 for sites 1, 2 and 3 respectively. Strong readings, or high virus presence, are indicated by Ct readings below 20, with progressively lower virus presence indicated as the readings increase to above 35 when no appreciable TRV is deemed to be present i.e. negative.

In general, TRV was identified in both sites 1 and 3 by both methods, although not entirely in agreement. The weeds sampled at both sites were well established. At site 1 the soil bait test identified TRV in 40 of the 45 samples, while the weed assay identified 22 points out of the 45. The data from both weed and bait tests in Table 4 indicate that areas 4 to c.15/16 as having higher TRV presence and areas 30 to 36, though the virus is quite widespread. The level of 'risk', as defined by a strong (low reading) Ct value in site one is transferred in Appendix 1 onto the GPS map 1 on a 0 to 3 basis, with 0 being no risk (reading 35-40), 1 (30 to 34.9), 2 (20 to 29.9) and 3 (at less than Ct reading 19.9) being high risk, in red for the weed assay and blue for the glasshouse bait test. Site 3 was slightly the reverse of site 1, with the weed assay identifying TRV in 30 of the 44 points and the glasshouse bait test identifying fewer points with detectable TRV, in 14 of the 44 points. These points are on a scaled GPS map 3 given in appendix 1, again with red for the weed assay and blue for the glasshouse bait test.

The site 2, at Brechin, demonstrated fewer positive results, with the weed assay identifying some low levels of TRV in 9 out of 45 points but the glasshouse bait test identifying only 2 points out of the 45. There were no really strong positive results from this site in the weeds (Table 5). The glasshouse bait plants and also a further 25 weed samples from the site taken on 8 June 04 (5 weeks later) had no trace of infection. The TRV positive points are presented in Appendix 1; GPS map 2 as per the other maps.

The roots of a number of barley plants at site 3, Tayport near Dundee, were tested for the presence of TRV in the roots. Thirty-two of the 45 roots tested indicated some infection of TRV, mostly at low level, with one point indicating a higher TRV content.

The potential to use a new filter paper system developed recently by Whatman to stabilise RNA samples directly on prepared impregnated FTA filter paper was briefly examined with a view to an easy grower-based sampling method. This method could potentially allow growers to sample points throughout a field by directly placing root sap from weeds onto the filter paper which stabilises the viral RNA and the filter discs forwarded to the testing laboratory. Seven root samples were tested for TRV presence using the FTA method. All samples had been previously tested for TRV by conventional RNA extraction and RT-PCR using taqman. The sample previously identified as highly positive (Ct <20) tested positive from the direct sampling of the filter disk (Ct= 30). All other samples were negative.

Sample used for FTA test	Ct from RT-PCR using routine RNA extraction	TRV	Ct from RT-PCR from RNA recovered from FTA paper	TRV
Site 3 Barley sample 1	24	xx	39.75	_
Site 3 Barley sample 4	31	x	40.00	_
Site 3 Barley sample 10	31	x	37.06	_
Site 3 Barley sample 19	38	_	38.00	_
Site 3 Barley sample 24	37	_	40.00	_
Site 3 Barley sample 44	35	x	40.00	_
Site 3 Petunia sample 1	19	xxx	32.04	x

Discussion

The results presented confirm the presence of TRV in the weed populations at all sites, as would be expected given the known epidemiology of the virus. The nematodes will acquire the virus by feeding on infected roots of a variety of weeds. While losing the virus during moulting, the maturing nematodes undoubtedly re-acquire the virus by feeding on the infected weed roots within its soil environment. Direct statistical comparison of the field weed assay and the glasshouse bait test are not entirely appropriate at this point, a crude measure is a 'Measure of Association' comparing those results in agreement (both tests detecting presence or the absence of TRV) with those classes where the two tests disagree – absence in one and presence in the other – with a value of 1 indicating a very strong association between the classifications and a value of 0 indicating no agreement. The observed associations for the Ct values as presence (<35.0) or absence (>35.0) are 0.51 for site 1, 0.76 for site 2 and 0.48 for site 3.

However, such a comparison does not give the complete situation. Examining the GPS map with the 'risk' values for site 1 it is evident that TRV/viruliferous nematodes are dispersed across the site, with possible very high risk areas at points 2–7 and 11-16; also 30-32, 37-39 and 42-43.

Examining the distribution detected within site 2, there was little TRV/viruliferous nematodes detected. The Ct data indicates some infection at the top of the map over the area with points 15, 18, 25 and also in the area with points 21, 29, 30, 38 and 39 with a third area suggested by points 43 and 45. This was a spring-sown crop with small, poorly established weeds being sampled. It is of interest within this particular GPS map (site 2), based on a soil textural survey, that the distribution of the few positive points lie entirely within the lighter coloured areas of the map, those areas with a lighter sandier texture. It would be interesting to apply this technique further to see if a close relationship exists both across this particular field and also at other sites.

Within site 3, points 1 to c. 3 are known to SCRI staff to be high-risk areas and this is largely borne out by the observed results. The TRV infection appears patchily dispersed throughout the area, possibly with higher risk areas at points 1 to 3 including 14, also 6 to 10 including18 and also 34 to 35. The

weed assay within this site detected more than twice the number of points compared to the glasshouse bait test.

Given the short project timeframe it was not possible to identify 'perfect' sites – those sites with growers having winter-grown cereal crops planning to grow potatoes in 2005, particularly as many growers with a TRV problem will tend to avoid where possible growing potato crops in such land. The sites included in this project were one winter-grown crop and two spring-grown cereal crops (sites 2 and 3). However, TRV was successfully identified within the weeds at each site as well as by the glasshouse bait test method, with a degree of agreement between these. How well the identification of TRV by both methods relates to the level of spraing in the following potato crop is the important comparison, this was be carried out in 2005. The 2004 field results are summarised onto the field scale maps of the three fields presented in Appendix 1, and also in 'contour' maps in Appendix 3.

The barley crop was examined within one site (site 3, Tayport) and the majority exhibited low levels of virus present. How well the virus is distributed throughout the plant roots is not known. If it is not well distributed within the roots and does not transfer easily within the barley roots then this may reflect the unselective grazing nature of the nematode rather than representing a substantial source of viral inoculum.

General interim conclusions based on 2004 TRV detection

The weed and the soil bait tests appeared to work well within the project, identifying TRV in all three sites. The time of sampling is known to be important for the glasshouse soil bait test and possibly also for the weed assay, though following a number of weeks nematode activity after weed establishment this is probably less critical. The weed assay can be utilised at anytime within a rotation, as can the soil assay. A potential advantage of using the weed population to assess the level of TRV infection is that the weeds are *in situ* for a number of months acting as bait plants for a long period, while the soil test will rely to a degree on the position of the nematodes within the soil profile at the particular time of sampling. A further advantage is that the sampling of weeds / weed roots may be carried out on site by growers and mailed directly to testing laboratories with accompanying savings in costs.

Brief results using a recently developed FTA Card Whatman 'filter' type of system whereby virus RNA (as found in root sap) can be directly placed and stabilised on prepared filter papers, which could then be posted directly to laboratories for testing appeared to indicate that recover was possible and this has been reported elsewhere. Rogers & Burgoyne (2000) reported that RT-PCR has been successfully carried out on RNA processed on the FTA paper.

Experimental section 2005

Project Work in 2005

The three TRV infected sites were planted in collaboration with industry partners. As described previously in section 3, the three sites were 1) near Branston in the East Midlands, 2) near Brechin in Angus, Scotland, and 3) at Tayport, near Dundee. The 2 ha sites were sampled for weeds in April/early May 2004 in a 25m grid pattern to give about 45 samples. Soil samples were taken in the same grid pattern at each site for use in glasshouse bait tests to allow comparisons between tests. Roots of the weed bait plants and conventional bait plants (ex-glasshouse test) were tested for absence or presence of TRV using the established TaqMan test, and the results were plotted on maps of the sites (Appendix 1).

During 2005 the three sites – Branston, Brechin and Tayport – were planted to potato crops with two sites using Pentland Dell to assess the level of observed spraing symptoms. At the third site, near Brechin, the variety had to be changed due to a few dead potato cyst nematodes being identified in the pre-planting soil test, so the variety Pentland Javelin, with H1 *G. rostochiensis* resistance, was substituted as it is equally susceptible to expressing spraing symptoms. Plots of 8 or 10 plants from each of the 2004 sampling points were harvested into store, cut open at c. 5 mm slices and scored for the level of spraing symptoms.

2005 Results and key findings

The 2005 growing season was mild with drier conditions later in the season. The Branston site was irrigated while the other sites were not. A few sample points were 'lost' at the Branston site, where nematicide was applied to the edge of the field, affecting c. 5 of the 2004 points: 1,10,19, 28 & 37, so these are not included, see Branston map in Appendix 1 and 3. In autumn 2005 potato tubers were sampled and assessed at each of the points within the three sites. The summary results of the field tests in 2005 are presented in Annexes 2 and 3. There was a good agreement between the high virus areas and spraing at the sites, and between the areas identified as clear and no spraing symptoms. It appears, however, that at very low TRV virus levels, their detection, or non-detection, and also the resultant expression of low levels of spraing are subject to a number of factors, including seasonal environmental factors. This makes the prediction of areas of low levels of spraing (c. less than 5%) less reliable than those areas with high TRV virus.

The Brechin site had few positive samples in 2004 as indicated by both the weed and the glasshouse bait tests and there were few spraing symptoms in 2005 and those that were observed were not severe. The results from this site are presented, but note taken of the low levels of incidence of the virus or spraing. Results are presented in Appendix 2, mapped onto distribution/contour maps of virus levels & proportion of spraing symptoms in Appendix 3.

Discussion of results

The results of the 2005 potato plots harvested from the 3 fields are presented in Appendices 2 and 3. Appendix 2 gives a summation of the results at the individual sample points in the three sites. The table below give an indication of how the two tests appear to have performed in relation to correctly identifying, or not, the incidence of the virus and the observed incidence of spraing in the following crops at the three sites.

Numbers agreeing/disagreeing with weed or glasshouse bait test at each of the 3 different sites

	BRANSTON	%	TAYPORT	%	BRECHIN	%
Weed test agreeing with symptoms 2005	25	66	31	70	25	56
Weed test disagreeing with symptoms 2005	13		13		20	
Bait test agreeing with symptoms 2005	26	68	11	25	18	40
Bait test disagreeing with symptoms 2005	12		33		27	

Examining the results in the table above and in Appendix 2, there are a number of points to consider. For the three sites, for both tests, there are a number of 'mis-diagnosis' based on the spraing symptoms – marked as X 0 or X 1, indicating that no virus had been detected but few symptoms had been observed, or that low level of virus had been detected but no symptoms in the tubers the following year. Taking a 5.0% (0.05 proportion) spraing incidence as a reasonable cut-off as a level below which low virus level may not be detectable with current methods but few symptoms are observed (e.g. X 0 in appendix 1) or, if low virus in 2004 was detected but did not result in any observed symptoms on 2005 (e.g. X 1 in Appendix 2). Weak results in such categories – less than 5% infection or low virus in tests – are highlighted in red typeface in Appendix 2. Where a higher discrepancy is observed between the 2004 virus tests and the 2005 observed spraing symptoms, e.g. little virus detected but many spraing symptoms in 2005 or a lot of virus detected in 2004 and few or no spraing symptoms observed the results are highlighted in red.

At Branston: the weed test had 7 such mis-diagnoses, and the bait test 8 'mis-diagnoses'
At Brechin: the weed test had 12 such 'mis-diagnoses', and the bait test 17 'mis-diagnoses'
At Tayport: the weed test had 1 such 'mis-diagnosis', and the bait test 4 'mis-diagnoses'

It is doubtful that these weak results are truly 'mis-diagnoses', but rather relate to the accuracy/predictive limit of either tests.

Examining the other results within Appendix 2,

Branston:	Weed test: 25 correct, 6 incorrect	Bait test: 26 correct, 4 incorrect
Brechin:	Weed test: 25 correct, 6 incorrect	Bait test: 26 correct, 10 incorrect
Tayport:	Weed test: 31 correct, 12 incorrect	Bait test: 9 correct, 30 incorrect

From the results in Appendix 2, it would appear that both tests detected the presence of virus, with the weed test appearing slightly more accurate, principally at the Tayport site. Both tests detected high TRV risk areas. The detection of the virus using the bait test may be more accurate when used by technicians more frequently, as experience in sampling time of year / depth of soil sample will have an important role in its accuracy.

The distribution of virus and the spraing symptoms in the following year are presented in Appendix 3, with the more intense the degree of red colour indicating higher virus or spraing symptoms. Generally, the more intense/higher or 'hot' patches identified in 2004 appear to give more symptoms as might be expected. The areas with lower virus detected resulted in more variable observations, plus or minus symptom expression.

The detection of virus is certainly possible with both tests. TRV was successfully identified within the weeds at each site as well as by the glasshouse bait test method, with a degree of agreement between these tests. The 'weed' test appears possibly to be slightly more accurate, and potentially has the advantage of less skilled operator requirements and hence lower costs, so allowing higher levels of sampling.

The problem of low level virus infection areas and how the season environmental factors will enhance or suppress feeding/virus transmission in the crop remain, in that detection of low virus levels may not translate during the season into spraing symptoms.

Practical recommendations for growers

The project was completed in autumn/winter 2005 with the detailed sectioning of all tubers taken into store from the plots within the three sites. Both the weed and bait tests offer the possibility of plotting the distribution of TRV in fields, particularly areas with high levels of nematodes/virus. From the results in Tables 1 to 6 and the field maps in Appendix 1, it would appear that both tests detected the presence of virus, with the weed test appearing slightly more accurate, principally at the Tayport site. Both tests detected risk areas. The detection of the virus using the bait test may be more accurate when used by technicians more frequently, as sampling time of year/depth of soil sample will have a role in its accuracy.

The distribution of virus and the spraing symptoms in the following year, 2005, are presented in 'contour' maps in Appendix 3, with the more intense the degree of red colour indicating higher virus or spraing symptoms. Generally the more intense/higher or 'hot' patches identified in 2004 appear to give more symptoms as observed in the contour maps presented, as might be expected. The areas in which lower virus levels were detected resulted in more variable observations, plus or minus symptom expression.

The detection of virus is certainly possible with both tests. TRV was successfully identified within the weeds at each site as well as by the glasshouse bait test method, with a degree of agreement between these tests. The 'weed' test appears possibly to be slightly more accurate, but potentially has the advantage of less skilled operator requirements and hence lower costs, so allowing higher levels of sampling. Given the known patchy nature of TRV distribution in many, though not all, fields, it would

be advisable to use a number of samples per field, possibly down to 30 or 40 metre intervals. A 25-metre grid system was utilised in the present study.

The problems of low level virus infection areas and how particular seasonal environmental factors enhance or suppress feeding / virus transmission in the crop remain, in that detection of low virus levels may not translate during the subsequent season into spraing symptoms.

General conclusions

The weed and the soil bait tests appeared to work well within the project, identifying TRV in all three sites. The time of sampling is known to be important for the glasshouse soil bait test and possibly also for the weed assay, though after a number of weeks nematode activity after weed establishment this is probably less critical. The weed assay can be utilised at any time within a rotation, as can the soil assay. A potential advantage of using the weed population to assess the level of TRV infection is that the weeds are *in situ* for a number of months acting as bait plants for a long period, while the soil test will rely to a degree on the position of the nematodes within the soil profile at the particular time of sampling. A further advantage is that the sampling of weeds / weed roots may be carried out on site by growers and mailed directly to testing laboratories with accompanying savings in costs. TaqMan tests can then be completed in a matter of days rather than the month required for bait tests. The use of weeds as *in situ* bait plants instead of conventional bait plants has clear logistical and economic advantages, and seems from our results to be equally reliable if not better for the identification of TRV-infested fields. Whether the opportunity for increased density of sampling can be turned to practical advantage is so far unproven.

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Achievement of milestones

Experimental timetable with **Detailed Milestones/Deliverables** (with dates):

Year 1 within project

1) Identify three suitable TRV-infected sites. Early spring 2004. [**Milestone**]
ACHIEVED by end April 2004

2) Sample weed population on detailed grid at 25 m intervals (c. 45 samples/site) at sites, and test roots for TRV by TaqMan. TRV 'map' of 2 ha sites based on weed results. [**Milestone**]
ACHIEVED by mid-May 2004

3) Soil samples taken at three selected sites to match 25 m grid as in 2) for bait test combined with TaqMan [**Milestone**]
ACHIEVED by mid-August 2004

4) Where possible, sample crop plants at selected sites on same grid and test roots for TRV by TaqMan. **ACHIEVED by end August 2004**

5) Interim report to BPC at end of September 2004. [**Deliverable**]
ACHIEVED by 22 October 2004

[contacted BPC/Dr. M. Storey regarding c. 3-week extension to write]

6) Plant tuber plots at three identified sites in 2005 **ACHIEVED by 30 April 2005**

7) Harvest plots into store and score for presence/absence of spraing. **ACHIEVED by 30 Jan 2006**

8) Report to BPC end of Jan 2006. [**Deliverable**]
ACHIEVED by 15 February 2006

9) Revised report to BPC end of April 2006

Acknowledgements

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TABLE 1. WEEDS SAMPLED AT SITE 1 NEAR BRANSTON, EAST MIDLANDS. SAMPLED 14/4/04

	Common name	Latin name	TRV
1	chickweed x3	Stellaria media	_
	willowherb x3	Epilobium hirsutum	
	groundsel x1	Senecio vulgaris	
2	groundsel x2	Senecio vulgaris	_
	chickweed x3	Stellaria media	
3	groundsel x4	Senecio vulgaris	_
	chickweed x1	Stellaria media	
4	groundsel x4	Senecio vulgaris	xx
	geranium x1	Geranium dissectum	
5	groundsel x4	Senecio vulgaris	_
	xchickweed x1	Stellaria media	
6	groundsel x4	Senecio vulgaris	xx
	xchickweed x2	Stellaria media	
7	groundsel x5	Senecio vulgaris	xx
	xchickweed x1	Stellaria media	
8	groundsel	Senecio vulgaris	_
9	groundsel x4	Senecio vulgaris	_
	xchickweed x1	Stellaria media	
10	groundsel x4	Senecio vulgaris	x
	fools parsley	Aethusa cynapium	
	grass x2	Poa annua	
11	groundsel x5	Senecio vulgaris	xx
	goosegrass x1	Galium aparine	
12	groundsel	Senecio vulgaris	xx
13	groundsel x4	Senecio vulgaris	xx
	chickweed x1	Stellaria media	
14	groundsel	Senecio vulgaris	xx
15	groundsel x4	Senecio vulgaris	xx
	xchickweed x1	Stellaria media	
16	groundsel	Senecio vulgaris	_
17	groundsel	Senecio vulgaris	_
18	groundsel x2	Senecio vulgaris	_
	goosegrass x1	Galium aparine	
	chickweed	Stellaria media	
19	groundsel x4	Senecio vulgaris	_
	wheat x1		
20	groundsel x4	Senecio vulgaris	_
	chickweed x1	Stellaria media	
21	groundsel x3	Senecio vulgaris	x
	chickweed x2	Stellaria media	
22	groundsel x3	Senecio vulgaris	_
	chickweed x2	Stellaria media	
23	groundsel	Senecio vulgaris	_
24	groundsel	Senecio vulgaris	x
25	groundsel	Senecio vulgaris	x
26	groundsel	Senecio vulgaris	_
27	groundsel	Senecio vulgaris	x
28	groundsel	Senecio vulgaris	_
29	groundsel	Senecio vulgaris	_
30	groundsel	Senecio vulgaris	_
31	groundsel	Senecio vulgaris	xx
32	groundsel	Senecio vulgaris	xx
33	groundsel	Senecio vulgaris	x
34	groundsel	Senecio vulgaris	x
35	groundsel	Senecio vulgaris	x
36	groundsel	Senecio vulgaris	xx
37	groundsel	Senecio vulgaris	_
38	groundsel	Senecio vulgaris	_
39	groundsel	Senecio vulgaris	x
40	groundsel	Senecio vulgaris	_
41	groundsel	Senecio vulgaris	_
42	groundsel	Senecio vulgaris	xx
43	groundsel	Senecio vulgaris	_
44	groundsel	Senecio vulgaris	x
45	groundsel	Senecio vulgaris	_

TRVlevel
<20 xxx
>20<30 xx
30 -35 x

Strong +ve indicated by

_ =negative, + =positive, ++ = strongly positive

TABLE 2. WEEDS SAMPLED AT SITE 2, NEAR BRECHIN. SAMPLED ON 29 APRIL 2004

Sample	Common name	Latin name	Size	TRV	Sample	Common name	Latin name	Size	TRV
1	chickweed x5	Stellaria media	1xM, 4xS	_	26	chickweed x5	Stellaria media	M	_
2	chickweed x4	Stellaria media	M	_	27	chickweed x4	Stellaria media	L	x
3	chickweed x4	Stellaria media	L	_	28	chickweed x6	Stellaria media	2xM, 4xS	_
4	chickweed x3	Stellaria media	M	_	29	chickweed x9	Stellaria media	S	x
	forget-me-not	Myosotis arvensis			30	chickweed x9	Stellaria media	S	x
5	chickweed x3	Stellaria media	M	_	31	chickweed x6	Stellaria media	S	_
	red dead-nettle x1	Lamium purpureum			32	chickweed x4	Stellaria media	S	_
6	chickweed x3	Stellaria media	M	_		forget-me-not x1	Myosotis arvensis		
7	chickweed x5	Stellaria media	VS	_		hemp nettle x1	Galeopsis spp		
	mayweed x2	Matricaria spp			33	chickweed x5	Stellaria media	S	_
8	chickweed x2	Stellaria media	M	_	34	chickweed x5	Stellaria media	M	_
	forget-me-not	Myosotis arvensis			35	chickweed x4	Stellaria media	L	_
	mayweed x1	Matricaria spp			36	chickweed x4	Stellaria media	L	_
9	chickweed x4	Stellaria media	M	_	37	chickweed x7	Stellaria media	S	_
	red dead-nettle x1	Lamium purpureum			38	chickweed x5	Stellaria media	2xM 3xS	x
10	chickweed x4	Stellaria media	3xM, 1xS	_	39	chickweed x5	Stellaria media	L	x
11	chickweed x2	Stellaria media	L	_	40	chickweed x2	Stellaria media	L	_
	red dead-nettle x1	Lamium purpureum				hemp nettle x1	Galeopsis spp		
12	chickweed x4	Stellaria media	M	_	41	chickweed x6	Stellaria media	S	_
13	chickweed x3	Stellaria media	M	_		hemp nettle x1	Galeopsis spp		
14	chickweed x4	Stellaria media	M	_	42	chickweed x4	Stellaria media	L	_
15	chickweed x4	Stellaria media	M	_	43	chickweed x6	Stellaria media	4xL, 2xS	x
16	chickweed x4	Stellaria media	1xL, 3xM	_	44	chickweed x2	Stellaria media	L	_
17	chickweed x4	Stellaria media	1xL, 3xM	_	45	chickweed x4	Stellaria media	2xL, 2xM	_
18	chickweed x4	Stellaria media	M	_		fumitary x1	Fumaria officianalis		
19	chickweed x5	Stellaria media	3xM, 2xS	_	47	chickweed x9	Stellaria media	S	
20	chickweed x5	Stellaria media	2xL, 3xS	_	48	chickweed x4	Stellaria media	S	
	forget-me-not x1	Myosotis arvensis				forget-me-not x1	Myosotis arvensis		
21	chickweed x4	Stellaria media	3xM, 1xS	_	49	chickweed x5	Stellaria media	S	
22	chickweed x4	Stellaria media	M	_		fumitary x2	Fumaria officianalis		
23	chickweed x4	Stellaria media	S	_	50	chickweed x3	Stellaria media	S	
	hemp nettle x1	Galeopsis spp				fumitary x2	Fumaria officianalis		
24	chickweed x6	Stellaria media	4xM, 2xS	_	51	chickweed x6	Stellaria media	5xS, 1xM	

25	chickweed x5	Stellaria media	3xM 2xS	x		fumitary x1	Fumaria officianalis

TABLE 3. WEEDS SAMPLED AT SITE 3, TAYPORT, SAMPLED 11/5/04

Sample	Common name	Latin	Size	Trv	Sample	Common name	Latin	Size	Trv
1	fat hen x1	Chenopodium album		xxx	26	groundsel x2	Senecio vulgaris		_
	chickweed x3	Stellaria media	L			chickweed x2	Stellaria media		
2	pansy x1	viola tricolor		x	27	fat hen x1	Chenopodium album		x
	fat hen x2	Chenopodium album				chickweed x3	Stellaria media	L	
	chickweed x2	Stellaria media	VL		28	chickweed x4	Stellaria media	M	x
3	chickweed x3	Stellaria media	L	xx	29	groundsel x3	Senecio vulgaris		x
4	chickweed x3	Stellaria media	L	x		pansy x1	viola tricolor		
5	chickweed x3	Stellaria media	L	x	30	groundsel x1	Senecio vulgaris		_
6	chickweed x3	Stellaria media	L	xxx		fat hen x1	Chenopodium album		
7	chickweed x3	Stellaria media	L	x		chickweed x2	Stellaria media	M	
8	chickweed x3	Stellaria media	L	xx	31	groundsel x3	Senecio vulgaris		_
9	chickweed x3	Stellaria media	VL	xx		pansy x1	viola tricolor		
10	chickweed x3	Stellaria media	L	_		fat hen x1	Chenopodium album		
11	groundsel x2	Senecio vulgaris		x		chickweed x1	Stellaria media		
	chickweed x3	Stellaria media	L		32	groundsel x2	Senecio vulgaris		_
12	groundsel x2	Senecio vulgaris		x		pansy x2	viola tricolor		
	chickweed x2	Stellaria media	L			fat hen x1	Chenopodium album		
13	groundsel x2	Senecio vulgaris		x	33	groundsel x2	Senecio vulgaris		_
	fat hen x3	Chenopodium album				pansy x3	viola tricolor		
	chickweed x1	Stellaria media	L			fat hen x1	Chenopodium album		
14	fat hen x3	Chenopodium album		xx		chickweed x2	Stellaria media		
	chickweed x2	Stellaria media	L		34	groundsel x2	Senecio vulgaris		x
15	groundsel x1	Senecio vulgaris		x		pansy x1	viola tricolor		
	fathen x1	Chenopodium album				chickweed x1	Stellaria media		
	chickweed x2	Stellaria media	L		35	groundsel x3	Senecio vulgaris		_
16	groundsel x2	Senecio vulgaris		_		chickweed x1	Stellaria media		
	chickweed x2	Stellaria media			36	groundsel x3	Senecio vulgaris		_
17	groundsel x4	Senecio vulgaris		_		chickweed x1	Stellaria media		
	pansy x1	viola tricolor			37	groundsel x2	Senecio vulgaris		x
18	fat hen x1	Chenopodium album		xx		pansy x1	viola tricolor		
	pansy x2	viola tricolor				chickweed x2	Stellaria media		

Sample	Common name	Latin	Size	Trv	Sample	Common name	Latin	Size	Trv
	chickweed x2	Stellaria media			38	groundsel x1	Senecio vulgaris		_
19	chickweed x2	Stellaria media	VL	x		chickweed x2	Stellaria media	VL	
20	groundsel x2	Senecio vulgaris		x	39	groundsel x1	Senecio vulgaris		x
	chickweed x1	Stellaria media				pansy x1	viola tricolor		
21	groundsel x1	Senecio vulgaris		x		chickweed x2	Stellaria media	L	
	pansy x3	viola tricolor			40	groundsel x1	Senecio vulgaris		x
	fat hen x1	Chenopodium album				fat hen x1	Chenopodium album		
	chickweed x1	Stellaria media				pansy x1	viola tricolor		
22	groundsel x4	Senecio vulgaris		_		chickweed x1	Stellaria media		
	mayweed x1	Matricaria spp			41	groundsel x2	Senecio vulgaris		_
	chickweed x1	Stellaria media	L			pansy x2	viola tricolor		
23	groundsel x2	Senecio vulgaris		x		chickweed x2	Stellaria media		
	chickweed x2	Stellaria media			42	groundsel x1	Senecio vulgaris		_
24	groundsel x3	Senecio vulgaris		x		pansy x2	viola tricolor		
	pansy x1	viola tricolor				chickweed x1	Stellaria media	L	
25	groundsel x2	Senecio vulgaris		_	43	groundsel x1	Senecio vulgaris		x
	chickweed x1	Stellaria media				fat hen x1	Chenopodium album		
						chickweed x2	Stellaria media	L	
					44	groundsel x2	Senecio vulgaris		x
						fat hen x1	Chenopodium album		
						chickweed x2	Stellaria media		
							Strong +ve indicated by		
						TRVlevel			
						<20 xxx			
						>20<30 xx			
						30 -35 x			

TABLE 4. SITE 1, NEAR BRANSTON, EAST MIDLANDS. WEEDS VS PETUNIA (BAIT PLANTS). COLLECTED 14/04/04

Sample	Weed (Ct)	TRV level	Bait (Ct)	TRV level	Sample	Weed (Ct)	TRV level	Bait (Ct)	TRV level
1	38.98	_	29.39	xx	24	31.23	x	34.79	x
2	39.74	_	20.55	xx	25	34.81	x	35.30	_
3	36.84	_	19.84	xxx	26	39.76	_	35.25	_
4	28.92	xx	21.53	xx	27	33.83	x	20.68	xx
5	40.00	_	34.12	x	28	37.85	_	35.66	_
6	26.38	xx	18.28	xxx	29	37.86	_	34.10	x
7	24.61	xx	24.27	xx	30	36.13	_	23.41	xx
8	37.08	_	29.12	xx	31	26.80	xx	24.27	xx
9	40.00	_	16.54	xxx	32	21.35	xx	18.38	xxx
10	34.94	x	31.47	x	33	33.70	x	33.30	x
11	24.48	xx	18.24	xxx	34	34.54	x	36.18	_
12	28.70	xx	15.28	xxx	35	34.08	x	34.20	x
13	25.09	xx	16.88	xxx	36	24.51	xx	21.81	xx
14	22.96	xx	20.81	xx	37	37.37	_	21.99	xx
15	21.46	xx	19.42	xxx	38	35.99	_	23.01	xx
16	40.00	_	19.23	xxx	39	34.88	x	22.97	xx
17	40.00	_	33.03	x	40	40.00	_	21.13	xx
18	36.93	_	27.25	xx	41	36.11	_	32.97	x
19	40.00	_	25.34	xx	42	25.12	xx	17.98	xxx
20	40.00	_	30.61	x	43	38.27	_	20.46	xx
21	32.58	x	33.18	x	44	33.04	x	30.35	x
22	36.63	_	36.62	_	45	35.70	_	33.80	x
23	35.07	_	33.26	x				TRV level	
								<20 xxx	
								>20<30xx	
								>30-35 x	

TABLE 5. SITE 2 NEAR BRECHIN. WEEDS VS PETUNIA (BAIT PLANTS). (COLLECTED 29/4/04)

Sample	Weed (Ct)	TRV level	Bait (Ct)	TRV level	Sample	Weed (Ct)	TRV level	Bait (Ct)	TRV level
1	36.78	_	37.08	_	24	37.61	_	36.33	_
2	39.63	_	38.15	_	25	34.95	x	35.84	_
3	39.59	_	39.72	_	26	37.16	_	39.42	_
4	39.20	_	39.09	_	27	34.00	x	38.25	_
5	35.69	_	38.45	_	28	36.33	_	39.01	_
6	39.57	_	37.00	_	29	31.76	x	39.77	_
7	40.00	_	35.15	_	30	33.43	x	38.07	_
8	39.63	_	39.29	_	31	36.75	_	38.86	_
9	40.00	_	39.60	_	32	40.00	_	39.67	_
10	39.26	_	38.95	_	33	37.55	_	39.28	_
11	38.56	_	38.62	_	34	37.11	_	39.43	_
12	36.62	_	37.98	_	35	36.88	_	38.53	_
13	37.60	_	39.97	_	36	36.09	_	38.81	_
14	37.23	_	39.18	_	37	38.46	_	39.14	_
15	34.67	x	39.44	_	38	33.45	x	39.54	_
16	37.44	_	39.07	_	39	32.23	x	40.00	_
17	35.90	_	39.23	_	40	37.38	_	36.77	_
18	37.11	_	32.49	x	41	36.20	_	39.72	_
19	36.14	_	36.57	_	42	39.05	_	37.95	_
20	38.06	_	38.04	_	43	32.65	x	38.70	_
21	34.86	x	37.30	_	44	36.24	_	40.00	_
22	37.42	_	38.68	_	45	38.20	_	34.45	x
23	38.11	_	38.33	_					
								TRV level	
								<20 xxx	
								>20<30xx	
								>30-35 x	

TABLE 6. SITE 3, TAYPORT NEAR DUNDEE. WEEDS VS PETUNIA (BAIT PLANTS). [COLLECTED 11/5/04]

Plot no.	Weed (Ct)	TRV level	Bait (Ct)	TRV level	Barley	TRV level	Plot no.	Weed (Ct)	TRV level	Bait (Ct)	TRV level	Barley	TRV level
1	18.95	xxx	19.39	xxx	23.62	xx	24	34.83	x	38.54	_	36.68	_
2	34.07	x	36.41	_	33.64	x	25	39.78	_	40.00	_	31.30	x
3	21.30	xx	35.32	_	30.86	x	26	37.21	_	37.51	_	31.71	x
4	33.64	x	35.51	_	30.92	x	27	34.05	x	36.40	_	30.50	x
5	33.55	x	33.94	x	33.38	x	28	34.56	x	38.10	_	30.65	x
6	18.05	xxx	23.30	xx	34.26	x	29	34.38	x	37.65	_	34.39	x
7	34.98	x	23.26	xx	31.56	x	30	38.02	_	37.70	_	33.19	x
8	22.81	xx	19.00	xxx	31.65	x	31	35.52	_	35.42	_	34.25	x
9	22.23	xx	34.66	x	32.06	x	32	36.81	_	35.18	_	32.23	x
10	35.95	_	27.40	xx	30.50	x	33	36.19	_	37.75	_	32.66	x
11	33.78	x	38.14	_	32.35	x	34	34.93	x	19.05	xxx	33.18	x
12	33.90	x	28.12	xx	31.09	x	35	39.69	_	19.60	xxx	34.90	x
13	33.36	x	36.71	_	32.84	x	36	40.75	_	35.15	_	35.60	_
14	29.09	xx	36.51	_	32.00	x	37	31.42	x	37.01	_	31.58	x
15	30.27	x	36.91	_	33.24	x	38	35.84	_	32.90	x	33.64	x
16	35.17	_	35.46	_	34.78	x	39	34.58	x	35.15	_	30.09	x
17	39.61	_	40.00	_	37.07	_	40	32.62	x	35.40	_	33.00	x
18	29.86	xx	35.28	_	36.85	_	41	35.32	_	34.44	x	30.02	x
19	31.86	x	36.06	_	37.91	_	42	36.80	_	35.51	_	35.67	_
20	34.80	x	35.97	_	35.40	_	43	31.69	x	35.57	_	34.16	x
21	31.92	x	35.19	_	35.30	_	44	32.96	x	18.81	xxx	34.90	x
22	35.41	_	35.09	_	36.19	_	45	34.83	x	38.54	_	36.68	_
23	34.24	x	34.15	x	33.57	x							
												TRV level	
												<20 xxx	
												>20<30xx	
												>30-35 x	

APPENDIX 1

For reference: Field plots gps points mapped within the sites, presenting the results of the weed and also the glasshouse bait test conducted in 2004. For reference to annex 2 & contour maps in Annex 3. The 'contour' maps for each site in Appendix 3 are the same maps in different form to present the data for comparison with the prevalence of spraing symptoms in 2005 season.

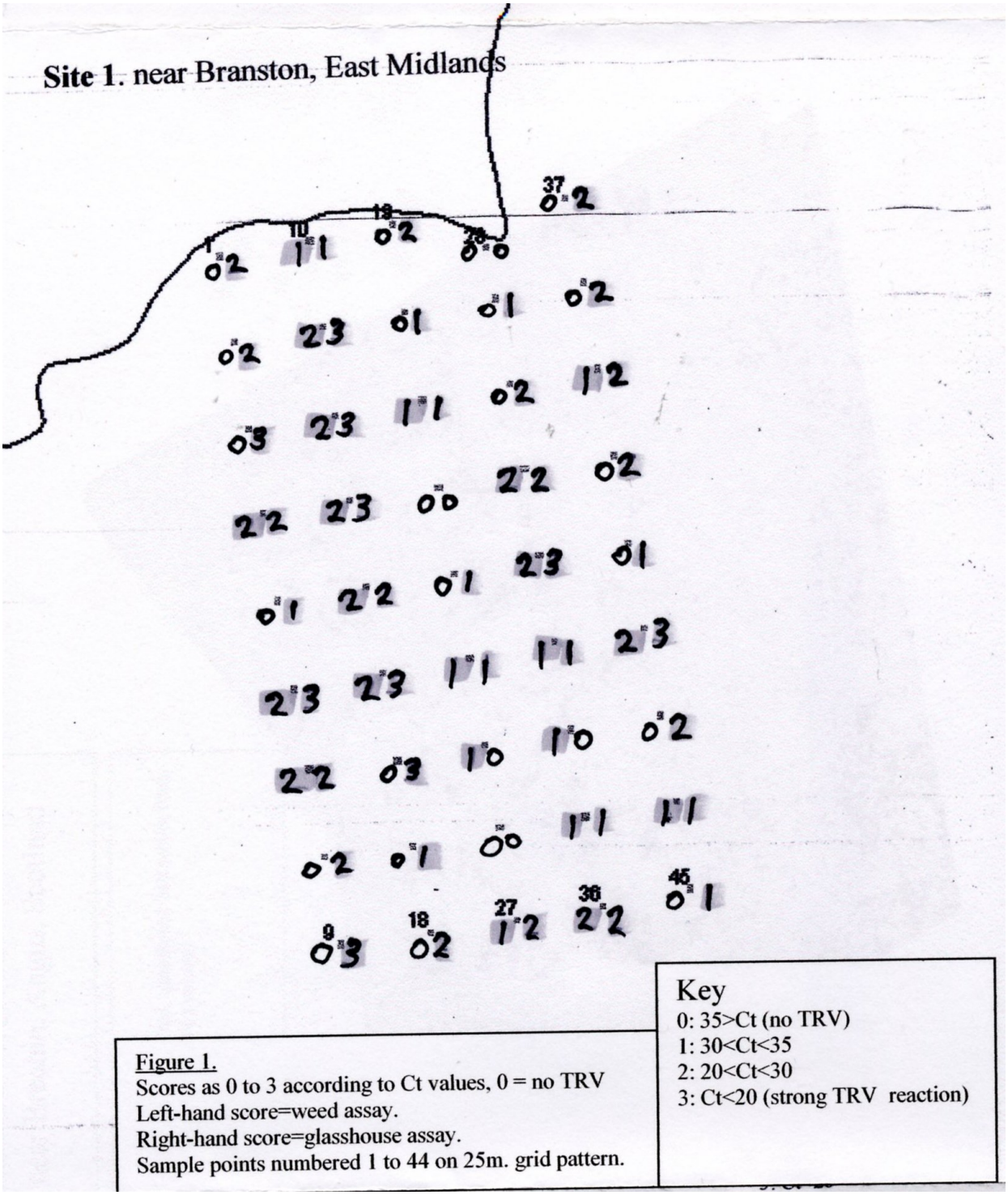
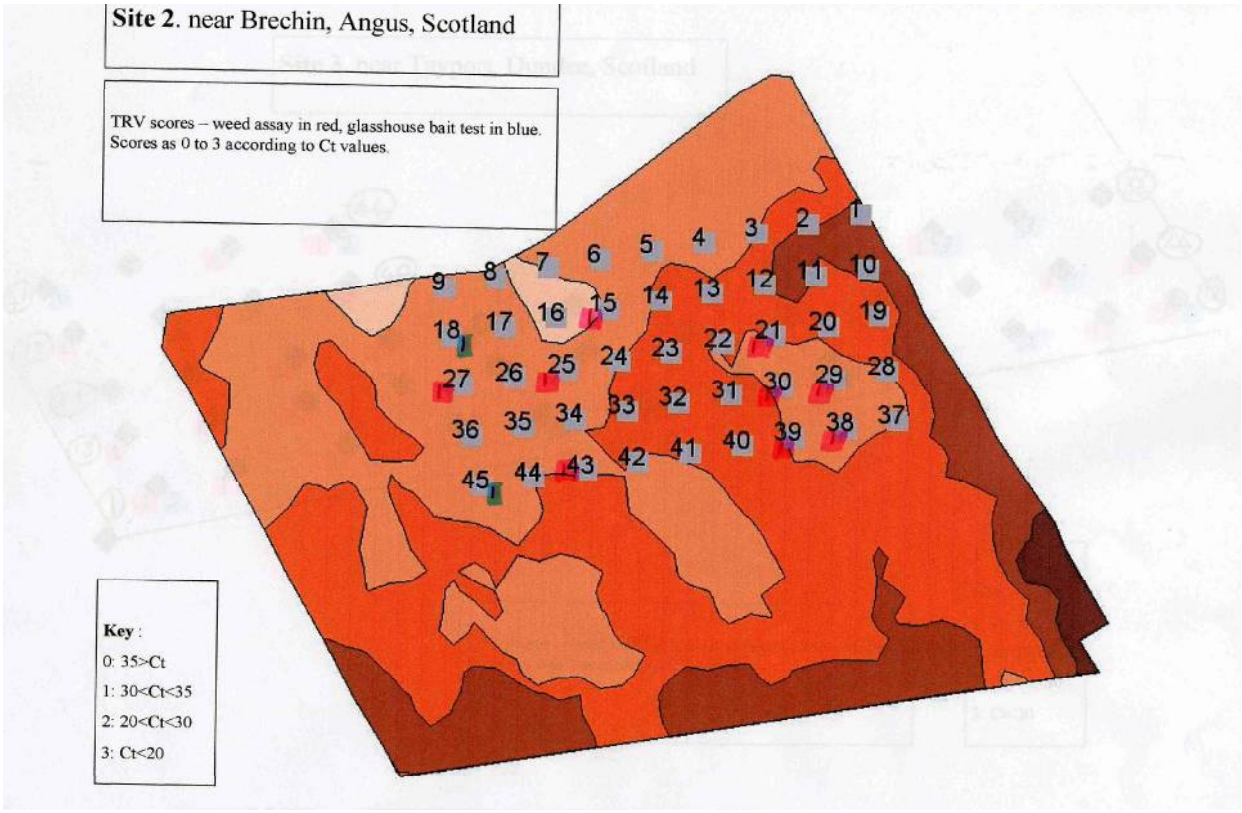


Figure 1.
Scores as 0 to 3 according to Ct values, 0 = no TRV
Left-hand score=weed assay.
Right-hand score=glasshouse assay.
Sample points numbered 1 to 44 on 25m. grid pattern.



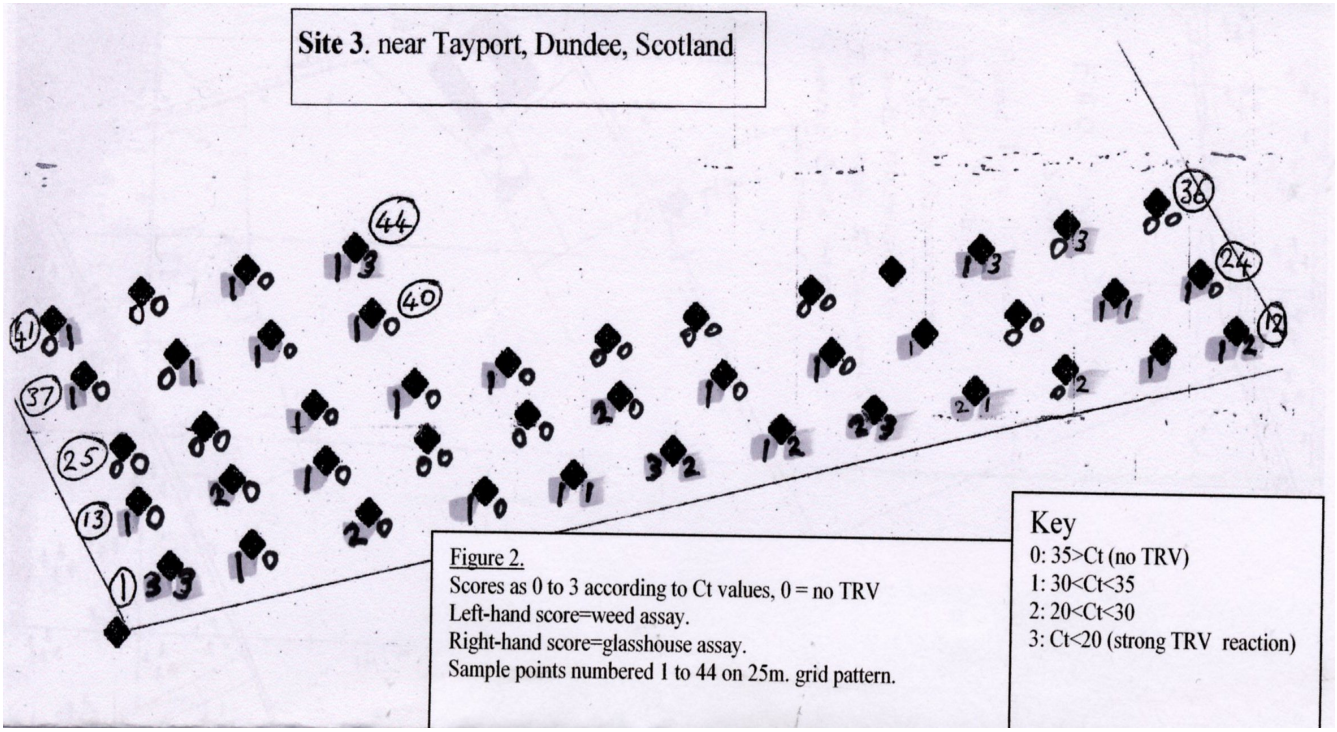


Figure 2.
Scores as 0 to 3 according to Ct values, 0 = no TRV
Left-hand score=weed assay.
Right-hand score=glasshouse assay.
Sample points numbered 1 to 44 on 25m. grid pattern.

APPENDIX 2

Results from 2005 potato plots

Numbers of tubers exhibiting spraing and total number of tubers at all sample points within each of the three sites. Proportion of tubers with symptoms presented (0.0 to 1.0).
Data for site 1 (Branston) in Table 1, site 2 (Brechin) in Table 2 and site 3 (Tayport) in Table 3.

The 'SCRI weeds 2004'and 'GH Bait 2004' columns have the scores 0 – no TRV to 3 –high TRV presence in 2004 as described in Appendix 1. In the same column, the assay results from 2004 report are presented for comparison with Y or X representing a correct or incorrect assessment in 2004 when compared to 2005 spraing results.

Where incorrect assessments appear in the tables 1 to 3 with the degree of symptoms not matching the levels of virus detected by either of the two detection methods in 2004, these results appear in red type. Those completely highlighted in red indicate instances where the 2004 glasshouse bait test or the weed bait test exhibit very poor agreement with the spraing symptoms observed in the tubers in 2005.

TABLE 7. BRANSTON SITE 2005 – SPRAING SYMPTOMS IN TUBERS 2005 AND ASSAY RESULTS 2004.

	BRANSTON SITE 2005						
Plot	Tubers w. TRV	Total tubers	Proportion w. Spraing	SCRI weeds 2004		G.H Bait 2004	
2	3	98	0.031	X	0	**Y**	**2**
3	30	96	0.313	X	0	**Y**	**3**
4	28	95	0.295	**Y**	**0**	**Y**	**2**
5	2	98	0.020	**Y**	**0**	**Y**	**1**
6	50	96	0.521	**Y**	**2**	**Y**	**3**
7	26	96	0.271	**Y**	**2**	**Y**	**2**
8	0	100	0.000	**Y**	**0**	X	2
9	0	100	0.000	**Y**	**0**	X	3
11	2	98	0.020	**Y**	**2**	**Y**	**3**
12	5	98	0.051	**Y**	**2**	**Y**	**3**
13	6	100	0.060	**Y**	**2**	**Y**	**3**
14	0	100	0.000	X	2	X	2
15	3	96	0.031	**Y**	**2**	**Y**	**3**
16	3	97	0.031	X	0	**Y**	**3**
17	0	100	0.000	**Y**	**0**	X	1
20	0	97	0.000	**Y**	**0**	X	1
21	0	100	0.000	X	1	X	1
22	0	100	0.000	**Y**	**0**	**Y**	**0**
23	0	99	0.000	**Y**	**0**	X	1
24	0	100	0.000	X	1	X	1
25	1	99	0.010	**Y**	**1**	X	0
26	0	100	0.000	**Y**	**0**	**Y**	**0**
29	0	100	0.000	**Y**	**0**	X	1
30	5	98	0.051	X	0	**Y**	**2**
31	52	99	0.525	**Y**	**2**	**Y**	**2**
32	33	95	0.347	**Y**	**2**	**Y**	**3**
33	3	100	0.030	**Y**	**1**	**Y**	**1**
34	0	92	0.000	X	1	**Y**	**0**
35	0	96	0.000	X	1	X	1
36	6	100	0.060	**Y**	**2**	**Y**	**2**
38	30	99	0.303	X	0	**Y**	**2**
39	58	100	0.580	**Y**	**1**	**Y**	**2**
40	6	94	0.064	X	0	**Y**	**2**
41	6	99	0.061	X	0	**Y**	**1**
42	9	98	0.092	**Y**	**2**	**Y**	**3**
43	0	100	0.000	**Y**	**0**	X	2
44	6	87	0.069	**Y**	**1**	**Y**	**1**
45	3	88	0.034	X	0	**Y**	**1**

Y = correct assessment of presence/absence of TRV when compared to spraing symptoms in 2005

X = incorrect assessment

Results underlined (in red) are those when assessment of degree of virus present in 2004 is not in good agreement with observed spraing symptoms in 2005.
Those highlighted indicate large disagreement.

TABLE 8. BRECHIN SITE 2005 – SPRAING SYMPTOMS IN TUBERS 2005 AND ASSAY RESULTS 2004

BRECHIN SITE 2005

Plot	Tubers w. TRV	Total tubers	Proportion w. Spraing	SCRI weeds 2004		G.H Bait 2004	
1	3	58	0.05	X	0	X	0
2	6	54	0.11	X	0	X	0
3	0	59	0.00	**Y**	**0**	**Y**	**0**
4	0	40	0.00	**Y**	**0**	**Y**	**0**
5	0	58	0.00	**Y**	**0**	**Y**	**0**
6	3	60	0.05	X	0	X	0
7	9	65	0.14	X	0	X	0
8	0	59	0.00	**Y**	**0**	**Y**	**0**
9	0	36	0.00	**Y**	**0**	**Y**	**0**
10	3	64	0.05	X	0	X	0
11	0	26	0.00	**Y**	**0**	**Y**	**0**
12	0	49	0.00	**Y**	**0**	**Y**	**0**
13	2	40	0.05	X	0	X	0
14	0	48	0.00	**Y**	**0**	**Y**	**0**
15	0	56	0.00	X	1	**Y**	**0**
16	0	47	0.00	**Y**	**0**	**Y**	**0**
17	0	52	0.00	**Y**	**0**	**Y**	**0**
18	0	35	0.00	**Y**	**0**	X	1
19	4	50	0.08	X	0	X	0
20	3	58	0.05	X	0	X	0
21	1	54	0.02	**Y**	**1**	X	0
22	0	70	0.00	**Y**	**0**	**Y**	**0**
23	0	60	0.00	**Y**	**0**	**Y**	**0**
24	0	58	0.00	**Y**	**0**	**Y**	**0**
25	3	62	0.05	**Y**	**1**	X	0
26	3	49	0.06	X	0	X	0
27	2	72	0.03	**Y**	**1**	X	0
28	0	66	0.00	**Y**	**0**	**Y**	**0**
29	2	63	0.03	**Y**	**1**	X	0
30	1	37	0.03	**Y**	**1**	X	0
31	1	70	0.01	X	0	X	0
32	2	74	0.03	X	0	X	0
33	2	66	0.03	X	0	X	0
34	4	65	0.06	X	0	X	0
35	3	60	0.05	X	0	X	0
36	3	46	0.07	X	0	X	0
37	15	75	0.20	X	0	X	0
38	5	80	0.06	**Y**	**1**	X	0
39	6	73	0.08	**Y**	**1**	X	0
40	4	76	0.05	X	0	X	0
41	11	80	0.14	X	0	X	0
42	0	80	0.00	**Y**	**0**	**Y**	**0**
43	0	63	0.00	X	1	**Y**	**0**
44	0	52	0.00	**Y**	**0**	**Y**	**0**
45	0	59	0.00	**Y**	**0**	X	1

Y = correct assessment of presence/absence of TRV when compared to spraing symptoms in 2005.
X = incorrect assessment.

Results underlined (in red) are those when assessment of degree of virus present in 2004 is not in good agreement with observed spraing symptoms in 2005.

TABLE 9. TAYPORT SITE 2005 – SPRAING SYMPTOMS IN TUBERS 2005 AND ASSAY RESULTS 2004

TAYPORT SITE 2005

Plot	Tubers w. TRV	Total tubers	Proportion w. Spraing	SCRI weeds 2004		G.H Bait 2004	
1	41	70	0.59	**Y**	**3**	**Y**	**3**
2	16	78	0.21	**Y**	**1**	X	0
3	22	75	0.29	**Y**	**2**	X	0
4	2	79	0.03	**Y**	**1**	X	0
5	34	70	0.49	**Y**	**1**	**Y**	**1**
6	19	69	0.28	**Y**	**3**	**Y**	**2**
7	23	64	0.36	**Y**	**1**	**Y**	**2**
8	7	70	0.10	**Y**	**2**	**Y**	**3**
9	10	65	0.15	**Y**	**2**	**Y**	**1**
10	0	50	0.00	**Y**	**0**	X	2
11	1	71	0.01	**Y**	**1**	X	0
12	2	66	0.09	**Y**	**1**	**Y**	**2**
13	22	65	0.34	**Y**	**1**	X	0
14	26	76	0.34	**Y**	**2**	X	0
15	24	73	0.33	**Y**	**1**	X	0
16	4	75	0.05	X	0	X	0
17	23	76	0.30	X	0	X	0
18	35	75	0.47	**Y**	**2**	X	0
19	14	71	0.20	**Y**	**1**	X	0
20	37	78	0.47	**Y**	**1**	X	0
21	11	68	0.16	**Y**	**1**	X	0
22	33	69	0.48	X	0	X	0
23	50	68	0.74	**Y**	**1**	**Y**	**1**
24	1	78	0.01	**Y**	**1**	X	0
25	32	75	0.43	X	0	X	0
26	31	74	0.42	X	0	X	0
27	42	66	0.64	**Y**	**1**	X	0
28	24	71	0.34	**Y**	**1**	X	0
29	12	75	0.16	**Y**	**1**	X	0
30	18	70	0.26	X	0	X	0
31	7	79	0.09	X	0	X	0
32	19	76	0.25	X	0	X	0
33	29	75	0.39	X	0	X	0
34	26	79	0.33	**Y**	**1**	**Y**	**3**
35	53	65	0.82	X	0	**Y**	**3**
36	17	76	0.22	X	0	X	0
37	3	69	0.04	**Y**	**1**	X	0
38	9	78	0.12	X	0	**Y**	**1**
39	21	56	0.38	**Y**	**1**	X	0
40	28	71	0.39	**Y**	**1**	X	0
41	20	71	0.28	**Y**	**1**	X	0
42	7	68	0.10	X	0	X	0
43	21	59	0.36	**Y**	**1**	X	0
44	26	78	0.33	**Y**	**1**	X	0

Y = correct assessment of presence/absence of TRV when compared to spraing symptoms in 2005
N = incorrect assessment.

Results underlined (in red) are those when assessment of degree of virus present in 2004 is not in good agreement with observed spraing symptoms in 2005.
Those highlighted indicate large disagreement.

APPENDIX 3

Contour maps of distribution of virus according to weed assay 2004, Glasshouse assay 2004 and spraing symptoms as observed a year later in 2005 at each of the 3 sites Branston, Brechin and Tayport.

Plot layout of the contour lines in 2005 relate to the level of TRV detected in plots as sampled in 2004 (as 'mapped' in Appendix 1), with the Y axis – 1 to 8 in Branston, 1 to 9 at Brechin and 1 to 12 in Tayport corresponding to the maps of the bait / taqman in the 2004 tests of the correspondingly numbered field plots and progressively across the lines of samples in the field as in the 2004 'maps'.

The X axis of the 3 maps represent the sample lines across the fields.

Site 1. At the Branston site, '1' being the samples 2 to 9, '2' being samples 11 to 18, '3' being samples 20 to 27, '4' being samples 29 to 36 and '5' being samples 38 to 45 at line 5 on the graph.

Site 2. At the Brechin site, '1' being the samples 1 to 9, '2' being samples 10 to 18, '3' being samples 19 to 27, '4' being samples 28 to 36 and '5' being samples 37 to 45 at line 5 on the graphs.

Site 3. At the Tayport site, '1' being the samples 1 to 12, '2' being samples 13 to 24, '3' being samples 25 to 36, '4' being samples 37 to 40 and '5' being samples 41 to 44 at line 5 on the graphs.

Note: The colour scale from 'white' through to 'red' in the following contour maps reflects increasing intensity of either TRV detection in 2004 maps or increasing incidence of spraing symptoms in the harvested 2005 potato tubers. The 2005 spraing symptom maps (centre map of each site in following pages) scale from 0 to 0.50 at Branston, from 0 to 0.175 at Brechin where there were far fewer symptoms noted and 0.0 to 0.80 at Tayport, where a high proportion of tubers expressed symptoms in some of the plots – deep red in colour.

The scales for the 2004 glasshouse bait tests and the weed bait test also reflect increasing levels of detection of the virus, with the scale relating to the taqman Ct values scales given in earlier tables, where 0 is no virus detected and 3 being a high level of virus detected with a Ct value of less than 20.

In 2004, at the Branston site significant trv levels were detected at some of the 45 points with the scale up to 2.5/3.0, while there was notably less virus detected at the Brechin site with reduced levels and the scale progressing up to 0.8 & 0.5 for the glasshouse bait test and the weed test respectively. The Tayport site recorded high levels of TRV in both the glasshouse and the weed tests with the scale increasing in levels to 2.5/3.0 in both tests.