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# Effects of tuber-borne M-type strain of tobacco rattle virus on yield and quality attributes of potato tubers of the cultivar Wilja

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**Abstract**

It was previously thought that Tobacco Rattle Virus (TRV) was self-eliminating from seed potato stocks and that the principal effects of the virus were the spraing symptoms (arcs or lines of corky brown tissue) formed in the tuber flesh. Recent work has clearly demonstrated that the virus can become fully and systemically established in some potato cultivars, with few, if any, tuber flesh symptoms. The studies reported here demonstrate that, in at least one such cultivar, an M-type strain of the virus can have a considerable effect on the growth and quality of the plant and its produce. When infected material was compared with healthy material, overall yield and yield components were severely affected by TRV, as were quality traits such as dry matter, after-cooking blackening and chemical components such as sugars, glycoalkaloids and chlorogenic acid. The results are discussed in terms of plant response to virus infection and plant protection mechanisms.

## Introduction

A number of tuber-borne potato viruses cause severe problems for commercial potato growers, whether the tubers are destined for seed, for processing, or for the fresh table market. Marshall et al. (1988) examined the effects of infection with Potato Leaf Roll Virus (PLRV) on four cultivars (Montana, Pentland Crown, Maris Piper and King Edward). They found an average yield reduction of 50%, which was attributable to less light being intercepted, to a lower efficiency with which the intercepted light was converted into dry matter, and to a smaller proportion of the dry matter being partitioned to the tubers. Hane and Hamm (1999) found that Potato Virus Y (PVY) infected plants of the cultivar Shepody produced 29% less yield in 1994, and 41% and 47% less total yield in two separate trials in 1995. In the cultivar Russet Norkotah, 46% and 49% total yield losses in 1994 and 1995 field trials were observed. The authors also reported that PVY-infected plants of both cultivars produced fewer and smaller tubers than did the control plants grown from virus-free seed.

With regard to soil-borne viruses, Nielsen and Mølgaard (1997) examined 23 potato cultivars in trials in Denmark in fields infested with Potato Mop-Top Virus (PMTV). They found that the occurrence of PMTV symptoms (spraing) did not influence total yield or dry matter content.

The work reported here examined the soil-borne virus Tobacco Rattle Virus (TRV) and its effect on the potato cultivar Wilja. TRV is transmitted by several species of *Paratrichodorus* and *Trichodorus* nematodes (Taylor and Brown, 1997). More than 100 plant species can be naturally infected, including a number of important crop plants (Harrison and Robinson, 1978), such as potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), gladiolus (*Gladiolus nanus* L.), tulip (*Tulipa fosteriana* L.), sugar beet (*Beta vulgaris* L.) and pepper (*Capsicum frutescens* L.). In potato, TRV causes spraing symptoms, which appear as arcs and lines of corky brown tissue formed in the tubers, rendering them unmarketable (Brown and Sykes, 1973).

Previously, it was thought that the primary effects of TRV were the visible spraing symptoms and that the

virus was generally self-eliminating from seed potato stocks. When tubers affected by spraing are planted, a few progeny plants develop 'stem mottle' symptoms in the foliage of a few of the resultant stems, though the proportion of affected stems is smallest in those cultivars that develop the most severe spraing symptoms in the tubers (Harrison, 1968). Recent work examining the epidemiology of the virus (Xenophontos et al., 1998) has identified some cultivars which can become systemically infected with M-type virus (possessing both RNA1 and RNA2 and producing nucleoprotein particles) while exhibiting few, if any, spraing symptoms in the tuber flesh. The virus could be maintained through a number of generations of vegetative propagation and these plants could act as sources for acquisition of the virus by trichodorid nematodes.

Pathogen infection is known to increase sugar levels in plants and such increases are associated with resistance (Horsfall and Dimond, 1957). Gene expression regulated by sugars is thought to be a response to developmental and environmental changes and expression of a number of pathogenesis-related proteins has been identified as being induced by sugars (Herbers et al., 1996; Tsukaya et al., 1991). Nolte et al. (1993) found that elevated chlorogenic acid levels were associated with tuber tissues in response to pathogen invasion and also as part of the wound healing process. McMillan et al. (1969) found an inverse correlation within eight potato cultivars between chlorogenic acid content and resistance to PLRV. However, Lyon and Barker (1984), using different potato cultivars, found no correlation between chlorogenic acid concentration and the inherent plant resistance to PLRV.

The work reported here examines the effect of systemic M-type TRV infection, not only on yield but also on a number of other economically important characteristics in the cultivar Wilja, including chlorogenic acid and sugar contents. The results are discussed in relation to the epidemiology of the virus and also to the host reaction to the virus.

## Methods and materials

### *Initial tuber inoculation*

Sandy soil of the Panbride series, containing large numbers of viruliferous *Paratrichodorus pachydermus* was obtained from a farm at Tentsmuir near Tayport, Fife, Scotland. The virus had previously been identified as a member of the PRN serotype of TRV (Robinson and Dale, 1994). The field soil was placed in 1400 $cm^3$ pots and tested with *Nicotiana tabacum* White Burley bait seedlings (Dale and Solomon, 1988). Pots, in which the bait seedlings expressed symptoms, were planted with virus-free Wilja seed tubers to provide TRV-infected seed tubers. Infection was subsequently confirmed by ELISA. This material formed the basis of the results reported by Xenophontos et al. (1998) from two vegetative generations.

The seed tuber material of cultivar Wilja, derived from this earlier study, was subsequently propagated through a further three vegetative generations (3 years) with the virus being passed to the daughter tubers in each year. Consequently, the material grown in the field in the present study had been grown previously over a total of five generations since infection with TRV.

The presence of TRV in the infected stock and its absence in the uninfected control stock was confirmed by ELISA on the foliage and RT-PCR on representative symptomless daughter tubers from each stock. The plants were also assessed using ELISA to ensure that neither PLRV nor PVY had entered the stocks.

### *Field trial*

Following a total of five consecutive vegetative cycles, there was sufficient field-grown seed tuber material to place in a replicated field trial, with eight replicates of TRV-infected Wilja and eight replicates of uninfected Wilja in a randomised complete block design. Each replicate consisted of fourteen plants, planted in a single row at ca. 40 cm spacing within rows, ca. 80 cm between rows and with guard rows at the edges of the trial. The trial was grown at Mylnefield, Dundee, UK, which is a carpous soil, described as a free-draining, sandy loam. The trial was planted on 6 May and harvested on 25 September, 1998.

### *Tests for presence of virus*

i. *Enzyme-linked immunosorbent assay (ELISA)*. Leaf samples were tested for the presence of TRV by indirect double antibody sandwich ELISA (Barbara and Clark, 1982) with an antiserum raised against TRV strain PRN.
ii. *Reverse transcription-polymerase chain reaction (RT-PCR)*. Tuber samples (ca. 1 $cm^3$) were taken from freshly cut tubers. Nucleic acid was extracted by the method of Barker et al. (1993). Reverse transcription and PCR were done as described by

Robinson (1992), except that 27 PCR cycles were done with an annealing temperature of 60 °C.

*Phenotypic assessment of tuber characters*

After harvest, the produce was size graded and assessed. Two tubers from the 45–65 mm size grade were stored for 111 days at ca. 8 °C. The two tuber samples from each replicate were then taken from storage, cut into halves and 3 slices from each tuber fried as described by Mackay et al. (1990). Each fried sample was visually assessed on a 1 (extremely dark) to 9 (extremely pale) scale.

A separate two-tuber sample from the 45–65 mm size grade was stored for 111 days at ca. 8 °C. The material was steam cooked as described by Griffiths et al. (1992) and scored for after-cooking blackening in the cooked material on a 1 (poor) to 9 (good) scale.

Apart from the chemical properties mentioned subsequently, further characters were assessed including total yield, tuber numbers within size grades, tuber yield within size grades, dry matter, appearance, and the rate at which the foliage emerged (1, very slow, to 9, very rapid) following planting.

*Sampling for chemical analyses*

At the same time as samples were taken for fry colour assessment, tuber material was also taken for chemical analyses. Material was chopped into 2 cm cubes and immediately immersed in liquid nitrogen, then stored at −20 °C before freeze-drying. The dried samples were milled in a Glen Creston Retsch cyclone mill fitted with a 0.5 cm sieve and then stored at −20 °C.

*Sugars*

Sugars were extracted from freeze-dried samples using 80% aqueous ethanol at 55 °C. The constituent sugars, fructose, glucose and sucrose, were separated and quantified using a high performance liquid chromatography method, based on that of Tamate and Bradbury (1985), full details of which are given in Brown et al. (1990).

*Chlorogenic acid*

The chlorogenic acid content of the freeze-dried samples was determined colorimetrically by the sodium nitrite method (Griffiths et al., 1992) and the results expressed as mg per 100 g freeze-dried matter (mg per 100 g FDM).

*Glycoalkaloids*

Glycoalkaloids were extracted from freeze-dried samples using 2% aqueous acetic acid containing 0.5 g per 100 ml sodium bisulphate (Hellenäs, 1986). After centrifuging, individual glycoalkaloid concentrations were determined using a high performance liquid chromatographic method (Dale et al., 1993) based on that of Hellenäs (1986). The total glycoalkaloid (TGA) content was taken as the sum of the individual values for $\alpha$-solanine and $\alpha$-chaconine and expressed as mg per 100 g FDM.

*Test for true seed transmission of TRV*

Fruits were collected from plants in infected and uninfected plots and true seed extracted and stored. The true seed was then sown in seed pans on 8 April 1999 before transplanting into plant pacs (Synprodo Plant Pac Ltd., UK) on 5 May 1999. Leaf samples from fifteen seedlings from each of the eight infected plots and from twenty seedlings from the uninfected plots were tested in batches of five for infection with TRV by ELISA. In addition, fifteen of the seedlings from infected plots were tested individually.

## Results

The plants grew well throughout the season with no obvious external adverse factors. Throughout the trial, the TRV-infected plots appeared markedly different. The plants were slower to establish than those in the control plots, and growth appeared to be slightly retarded or less vigorous. This can be seen in Figure 1 and confirmed in the assessment of plant establishment following emergence (scored on a 1 (poor) to 9 (good) scale) in Table 1. The produce of the TRV-infected plots appeared to be significantly affected by the virus, with almost all the tubers from the infected plots exhibiting a degree of secondary growth when compared to those from the uninfected plots (Figure 2). The results for tuber yield and number within the different size grades are presented in Figures 3 and 4, with appropriate standard error bars to allow within-size-grade comparisons. The distributions of yield and tuber number over the four size grades (< 35, 35–45, 45–65

*Figure 1.* Comparison of foliage establishment between a TRV-infected plot of cultivar Wilja (left) and a healthy control plot (right). Phenotypic assessment on a 1 (poor) to 9 (good) scale presented in Table 1.

and > 65 mm) were compared using the Kolmogorov–Smirnov two sample test (Conover, 1971). The test confirmed the results, apparent within Figures 3 and 4, that the distributions, both for yield and for tuber number, were significantly different between infected and uninfected plots (yield, Chi-squared, 2df = 16, $P < 0.005$; tuber number, Chi-squared, 2df = 352.77, $P < 0.005$). The produce of the TRV-infected plots was noticeably smaller, particularly when comparing the yield and number of tubers within the 45–65 mm grade. There was a significant increase in tuber numbers in the smallest size (< 35 mm) grade.

The effect of the virus on a number of tuber quality traits was also examined and the results are summarised in Table 1 with the appropriate statistics. With regard to table quality, TRV exacerbated the observed levels of after-cooking blackening, decreasing the mean visual score from 5.75 (acceptable) to 4.31 (unacceptable) and this was reflected in increased levels of chlorogenic acid, the principal causal agent of after-cooking blackening through the formation of a colourless ferrous ion complex which, on exposure to air, oxidises to

*Table 1.* Summary of data and statistics of variates assessed

| | Uninfected | Infected | LSD | Probability |
|---|---|---|---|---|
| Plant establishment (1 poor 9 good) | 7.37 | 2.50 | 0.695 | < 0.001 |
| Dry matter (%) | 21.77 | 18.85 | 1.281 | < 0.001 |
| Fry colour (1 poor 9 good) | 3.00 | 3.31 | 0.196 | 0.004 |
| After-cooking blackening (1 poor 9 good) | 5.75 | 4.31 | 0.534 | < 0.001 |
| Chlorogenic acid (mg/100 g FDM) | 78.41 | 116.16 | 11.701 | < 0.001 |
| Glucose (mg/100 g FDM) | 2.79 | 2.44 | n.s. | 0.209 |
| Fructose (mg/100 g FDM) | 0.85 | 0.87 | n.s. | 0.837 |
| Sucrose (mg/100 g FDM) | 0.42 | 0.56 | 0.043 | < 0.001 |
| TGA content (mg/100 g FDM) | 26.7 | 17.4 | 5.98 | 0.005 |
| $\alpha$-Solanine (mg/100 g FDM) | 11.79 | 8.33 | 3.145 | 0.033 |
| $\alpha$-Chaconine (mg/100 g FDM) | 14.93 | 9.10 | 3.042 | 0.001 |
| Solanine : Chaconine ratio | 0.782 | 0.934 | 0.1354 | 0.031 |

Mean values based on eight replicates TRV-infected material and eight replicates uninfected material for the potato cultivar Wilja.
n.s.: Not significant.

*Figure 2.* Normal tubers from healthy control plot (right) compared with degree of secondary growth found in produce of TRV-infected plants (left).

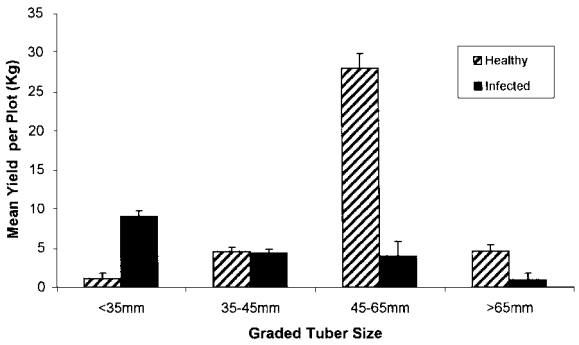


*Figure 3.* Comparison of mean plot yields (kg) within four size grades between TRV-infected and uninfected control plants.

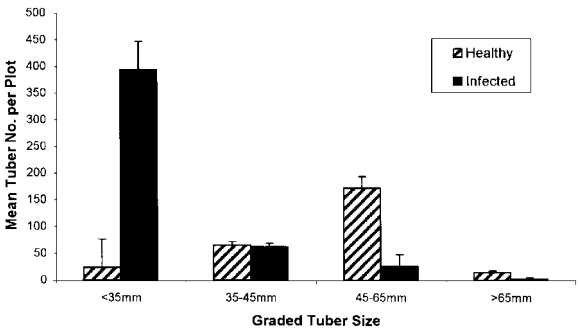


*Figure 4.* Comparison of mean plot tuber numbers within four size grades between TRV-infected and uninfected control plants.

a bluish-grey ferric ion based complex (Griffiths et al., 1992).

With regard to the processing attributes of the material, it should be noted that, while cultivar Wilja can be used for French fry production, it is not normally regarded as a high dry matter processing cultivar. The fry colour score of the TRV-infected material was slightly increased (paler) compared to the uninfected control material, although not to an acceptable level (ca. 5.0 fry colour score). Even so, the dry matter content of the infected material was reduced by almost 3.0% and this would certainly be regarded as a decrease in processing quality.

The effect of TRV infection on glycoalkaloid levels – an important aspect of nutritional quality – is also presented in Table 1. It would appear that the infected material had slightly reduced glycoalkaloid levels. TRV infection resulted in a small but statistically significant reduction in the concentration of both $\alpha$-solanine and $\alpha$-chaconine. Notably, the ratio of $\alpha$-solanine to $\alpha$-chaconine changed from 0.782 to 0.934, reflecting the fact that TRV infection appeared to have a greater effect on $\alpha$-chaconine concentration, which was reduced by almost 40% in the infected tubers as compared with a 30% reduction in $\alpha$-solanine content.

TRV was not detected in any of 120 seedlings grown from seed collected in the infected plots.

## Discussion

These results are the first reports that persistent, symptomless infection of potatoes by an M-type TRV isolate has significant effects on the growth of the plants and also, importantly, on various quality attributes. Previous work on TRV infection focused on plants showing clear, or classical, spraing symptoms (Cadman, 1959; Harrison, 1968). In potato cultivars which show spraing symptoms, the principal effects on the product are the visible arcs/necrotic marks within the tuber flesh which, even at relatively low levels (ca. 5–10%), can result in entire crops being rejected at the point of sale. Such symptoms are produced in a number of cultivars in which the TRV infection does not become fully systemic and the virus isolates are usually of the NM type (RNA1 only) (Harrison and Robinson, 1982)

The elucidation of the epidemiology of TRV in relation to potatoes (Xenophontos et al., 1998) has identified potato cultivars that can become fully systemically infected with M-type virus while exhibiting few, if any, symptoms in the tuber flesh. These cultivars may, perhaps, be more appropriately described as truly susceptible to infection. The results reported here indicate that, in at least one such cultivar, once TRV is fully established systemically, it can have a significant and detrimental effect on a number of yield components and important quality attributes. The most evident phenotypic effects are a significant decrease in tuber size, accompanied by a large increase in tuber number (Figure 4) and also a notable degree of secondary growth resulting in misshapen tubers to the extent that much of the produce of infected plants would be unsaleable (Figure 2). Taking into account the notable effect of the M-type TRV infection on emergence and canopy development, it would appear reasonable to suggest that the virus affects the efficiency of interception of solar radiation, the conversion into dry weight, and the subsequent partitioning of dry weight to the tubers, ultimately contributing to reduced final yield. The dramatic reduction in tuber size, coupled with an equally dramatic increase in tuber number, are indicative of a degree of stress within the TRV-infected plants. This is further reflected in the dry matter comparison (Table 1) between TRV-infected and uninfected material, in which the DM content is reduced, possibly reflecting the significantly increased number of sinks within the plant in the form of tubers initiated but with a restricted supply of dry matter from the source or canopy.

The significant increase in chlorogenic acid content of the TRV-infected material, from 78 to 116 mg/100 g FDM (Table 1), is a notable observation. Phenolic compounds such as chlorogenic acid are believed to be an important component of the general defence mechanism of many plants to infection (Friend, 1985). There is contradictory evidence within the literature concerning any inhibitory effects of chlorogenic acid on microbial growth, which is fully reviewed by Lyon (1989). In the TRV-infected material studied, the increased levels of chlorogenic acid appear to be associated with the virus infection and appear, from observation, to be quite evenly distributed throughout the tuber flesh. Dinkle (1964) demonstrated an association between chlorogenic acid and physiological internal necrosis in potato tubers. Whether the levels of chlorogenic acid in the TRV-infected material are due to a plant defence response or to a wound healing response is unclear.

Another factor associated with plant defence responses may be the observed increase in sucrose levels. Tadege et al. (1998) found increased sucrose levels in leaf tissue in response to *Phytophthora infestans* invasion compared to the control uninfected plants, suggesting that sugar metabolism played an important role in the process of programmed cell death in plants as found in the hypersensitive response. The changes to sucrose levels found in the TRV-infected tubers may be associated with resistance to infection or with changes to carbohydrate synthesis.

Transmission of TRV through seed of potatoes that were also infected with the NTN strain of PVY has been reported (Horváth et al., 1996), but we obtained no evidence for transmission of TRV through true potato seed.

The studies reported here have identified a range of changes in both phenotype and chemical composition within tuber tissue, attributable to infection by the M-form of TRV. Not only does the virus have a significant detrimental effect on yield and yield components, but also on tuber quality. The systemic infection of such true susceptible potato cultivars also initiates a metabolic response within the plant cells, which manifests through the observed changes to the levels of important components such as chlorogenic acid, sugars and glycoalkaloids. These observed changes are

important in terms of degree of susceptibility, plant defence reactions, and in terms of the degree of, or lack of, hypersensitive response observed within the tuber flesh.

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