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Factors affecting the development of disease symptoms in potatoes infected by *Tobacco rattle virus*

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Abstract

Infection of potato plants with *Tobacco rattle virus* by its nematode vector can have different outcomes, including the development of spraing symptoms in progeny tubers. A novel syndrome described here comprises a generalized mottle in leaves on all stems, together with virtually total failure to produce daughter tubers. The outcome of infection depends partly on the potato genotype, but field trials with 15 varieties showed that the incidence and severity of spraing symptoms at three sites differed. There was no evidence that this was due to differences in virulence among the virus isolates at the sites, but it was probably the result of environmental differences that influenced the numbers and activity of the vector nematodes. At the most severely affected site, spraing symptoms were found in all varieties tested, except Record, including several that were not affected at the other two sites. Taking both severity and incidence of symptoms into account, the ranking of varieties was similar at each site. The incidence of spraing symptoms was greater in larger than in smaller tubers, and increased with later harvest dates, but did not increase when early harvested tubers were stored. *Tobacco rattle virus* was detected in the roots of many of the weeds growing at one of the sites and in the roots of a few plants of the subsequent barley crop.

Abbreviations: NIAB – National Institute of Agricultural Botany; TRV – tobacco rattle virus.

Introduction

Spraing disease of potatoes is characterized by arcs and/or flecks of brown tissue in the flesh of affected tubers and is caused by infection by either *Tobacco rattle virus* (TRV) or *Potato mop-top virus*. TRV is transmitted by soil-inhabiting *Trichodorus* and *Paratrichodorus* (trichodorid) nematodes, which tend to be found in light free-draining soils. TRV is the principal cause of spraing disease on such soils. Maintenance of TRV at a site involves its repeated acquisition from and transmission to susceptible plant species by the local trichodorid population. Weed species are the main virus reservoirs in this process, but potato (and most other crop species) are unimportant (Cooper and Harrison, 1973). Because trichodorids are relatively static, TRV populations at different sites are isolated from one another and, consequently, the virus has evolved into many different strains. In M-type isolates, which contain both RNA-1 and RNA-2, some of this diversity can be recognized serologically and also in the association of different TRV isolates with distinct trichodorid species (Ploeg et al., 1992). However, neither of these tests can be applied to NM-type isolates, which contain only RNA-1 and do not produce nucleoprotein particles. TRV isolates also differ in the symptoms they induce in infected plants (Lister and Bracker, 1969; Harrison and Robinson, 1978). The assessment of varietal responses to TRV requires exposure to viruliferous nematodes, and such tests show that potato cultivars differ in their propensity to develop spraing symptoms. However, at present it is unclear whether or not the ability of a variety to produce spraing symptoms is entirely genetically determined. Conflicting reports in the literature on the susceptibility to spraing of particular cultivars, such as Bintje (e.g. Maas, 1975; Mojtahedi et al., 2001), suggest that there might be differences among virus isolates in their effects on potato cultivars. Alternatively, the incidence of spraing may be influenced by cultural and environmental factors, such as the previous cropping history of the site (Maas, 1975), the date at which the crop was lifted (Svensson, 1974), rainfall during the season (Cooper and Harrison, 1973) or soil temperature (van Hoof, 1975).

In this work, experiments were designed to investigate some of the factors, including differences among virus strains, that may affect the incidence of spraing disease.

Materials and methods

Variety field trials

In 2002, 15 potato varieties were grown at each of three sites in two-plant plots in a randomized block design with eight replicate plots, each trial occupying about 200 m^2 in total. After the foliage had senesced, tubers were harvested. Each tuber was sliced and scored for internal necrosis on a 1–9 scale. Scores of 1–4 represent typical spraing symptoms with the presence of corky tissue, 1 being the most severe and 4 the least. A score of 9 indicates complete freedom from symptoms, and 8 and 7 indicate the presence of one or a few brown spots, not typical of spraing. Scores of 5 or 6 represent intermediate degrees of necrosis of doubtful origin. Even in a heavily diseased crop, some tubers show no symptoms, probably because they have escaped infection. Symptom severity for each plot was therefore scored in a way that emphasizes the contribution from the most severely affected tubers, by using index ii of Dale and Solomon (1988), i.e. as the mean of individual tuber scores within three units of the lowest score in that plot. An overall score for each variety at each site was calculated from the plot scores in the same way. Incidence of spraing for each variety at each site was calculated as the percentage of tubers scored in classes 1–4. A combined score was calculated for each variety at each site as

$$9 - [(9 - \text{Symptom score}) \times \text{Relative incidence}]$$

where the relative incidence is the incidence for that variety divided by the greatest incidence for any variety at that site.

Glasshouse tests

The same 15 potato varieties were subjected to glasshouse tests as described by Dale and Solomon (1988). Soil was taken from within each of the three trial areas before the trial was planted and eight tubers of each variety were exposed to each soil in individual pots. The pots were arranged on the glasshouse bench in a randomized block design. After the foliage had died down, the daughter tubers were harvested from each pot and stored over winter at about 5 °C. In the following spring, each group of daughter tubers was grown on in the glasshouse in sterile compost. Soon after emergence, foliage samples, consisting of four leaflets from different stems, were tested for the presence of TRV by indirect double antibody sandwich ELISA (Barbara and Clark, 1982), using antiserum raised against TRV strain PRN for the tests in soil from sites A and B or against strain RQ for the test in soil from site C. The tubers were then recovered, sliced and scored for spraing symptoms. The score for each original nematode-exposed tuber was taken as that of the most severely affected daughter tuber, on the 1–9 scale described previously. The overall score for each variety in each soil was calculated as the mean of the lowest four original tuber scores.

Disease progress experiment

In a separate experiment at site C, 150 plants each of cvs Pentland Dell and Santé were grown in separate plots. One hundred days after planting and on three subsequent occasions at 2-week intervals, tubers from 30 randomly selected plants of each variety were lifted, washed and graded into larger (>45 mm) and smaller (<45 mm) classes. Each class was divided into four equal sub-samples. One sub-sample was sliced immediately and the number of tubers with spraing symptoms was recorded. The other sub-samples were stored in the dark at 15 °C for 2, 4 or 6 weeks before being examined.

RT-PCR

Nucleic acid was extracted from potato leaf samples by method B of Robinson (1992). Barley root samples were ground up with water in a Homex homogenizer (Bioreba) and nucleic acid was extracted by using a KingFisher total RNA purification kit (Thermo Labsystems). Reverse transcription and PCR were done as described by Robinson (1992), except that 30 PCR cycles were done with an annealing temperature of 50 °C .

Results

Observations on cv. Spey

Leaves on about 5% of plants in a commercial crop of potato cv. Spey growing near Montrose, Angus in 2000 were affected by mottle symptoms. On most of these plants, only one stem was affected and the symptoms were reminiscent of stem-mottle disease, which is caused by TRV (Rozendaal and van der Want, 1948). In others, the mottle was generalized and affected leaves on all stems. RT-PCR detected TRV in mottle-affected leaves from plants of both types, but sap extracts inoculated to *Chenopodium amaranticolor* induced scarcely any lesions, and none after freezing and thawing, which destroys the infectivity of NM-type isolates (Cadman, 1962). The TRV isolates infecting plants with both kinds of symptom therefore seemed to be of the NM type, as is usually the case in stem-mottle disease (Harrison and Robinson, 1982). Plants with generalized mottle bore few or no daughter tubers, but tubers were collected from other symptom-bearing plants and were grown on in the next year (2001) at Gourdie Farm, near Dundee. The resulting plants were of three types. Many were symptomless, and TRV was not detected in leaf samples by ELISA or by RT-PCR. Several were affected by a prominent mottle in leaves on one or two stems, and in these leaves TRV was detected by RT-PCR but not by ELISA, and virus particles were not observed by electron microscopy of negatively-stained extracts, implying that the infection was of the NM type. A few displayed symptoms similar to the leaf tip discolouration described by Xenophontos et al. (1998) as typical of potato plants systemically infected with TRV, and in these plants TRV was detected by both RT-PCR and ELISA, indicating that they contained M-type TRV. No spraing symptoms were found in daughter tubers on any of these plants.

Variety field trials

To examine the possibility that different strains of TRV might affect potato varieties differently, field trials were grown at three sites all of which had a history of TRV infection. At site A, at Tayport, Fife, and site B, at Bagthorpe, Norfolk, the virus isolates were serologically similar to strain PRN (Cadman and Harrison, 1959) and were transmitted by *Paratrichodorus pachydermus*, whereas the virus at site C, at Burrelton, Perthshire, was serologically similar to strain RQ (Robinson and Harrison, 1985) and was transmitted by *Trichodorus primitivus*. There was no evidence from serological or nematode transmission tests for the presence of more than one strain of the virus at any of the sites. Fifteen potato varieties were chosen to represent a range of reactions to TRV based on their classification in the *NIAB Descriptive List of Potatoes*. The results of these trials are summarized in Table 1. Maris Bard and Pentland Dell developed severe spraing symptoms at all three sites, although the percentage of tubers affected varied considerably between sites. Claret was also consistently affected, although both symptom severity and incidence were generally lower than in Maris Bard or Pentland Dell. At the other extreme, no tuber of Record was affected at any of the sites. Bintje, Hermes, Saturna and Saxon were affected only at site A, where the overall incidence of spraing was greatest, and even at this site the numbers of tubers affected were small. The remaining seven varieties were generally intermediate in both symptom severity and incidence. The ‘combined score’, while somewhat arbitrary, attempts to allow for differences in incidence between sites but to combine differences in incidence among varieties with differences in symptom severity. When this criterion was used to rank the varieties at each site, pairwise

Table 1. Spraing symptom score, % incidence (italic) and combined score (bold) for 15 potato varieties in field trials at three *Tobacco rattle virus*-affected sites, together with the results of glasshouse tests with soil from site A, and the varieties' rating for spraing in the *NIAB Descriptive List of Potatoes*

	Site A			Site B			Site C			Glasshouse tests (site A)	NIAB rating
Pentland Dell	1	*87.3%*	**1**	2	*18.3%*	**2**	1	*43.9%*	**2**	**2**	**1**
Claret	3	*55.6%*	**5**	3	*12.7%*	**5**	2	*29.4%*	**5**	**4**	**1**
Maris Bard	2	*91.2%*	**2**	2	*12.5%*	**4**	1	*52.2%*	**1**	**2**	**2**
Lady Balfour	4	*29.8%*	**7**	2	*11.2%*	**5**	4	*11.1%*	**8**	**5+**	**5**
Wilja	3	*24.9%*	**7+**[1]	4	*8.4%*	**7**	4	*10.7%*	**8**	**6+**	**5**
King Edward	3	*31.1%*	**7**	4	*1.4%*	**9**	5	*5.6%*	**9**	**7**	**6**
Nadine	3	*41.2%*	**6**	4	*7.4%*	**7**	5	*12.2%*	**8**	**3+**	**6**
Santé	4	*28.8%*	**7**	3	*8.2%*	**6**	5	*11.6%*	**8**	**5**	**6**
Shepody	3	*30.0%*	**7+**	4	*2.6%*	**8**	4	*5.9%*	**8**	**7+**	6
Bintje	3	*2.5%*	**9**	9	*0%*	**9**	3	*0.4%*	**9**	**9**	**7**
Romano	3	*29.1%*	**7**	4	*7.1%*	**7**	5	*0%*	**9+**	**7**	**7**
Saturna	4	*6.4%*	**9**	9	*0%*	**9**	9	*0%*	**9**	**8**	**7**
Saxon	3	*6.5%*	**9+**	8	*0%*	**9**	5	*0%*	**9**	**8+**	**7**
Hermes	2	*2.8%*	**9**	9	*0%*	**9**	9	*0%*	**9**	**9**	**8**
Record	9	*0%*	**9**	9	*0%*	**9**	9	*0%*	**9**	**9**	**8**

[1] + indicates varieties in which systemic TRV infection was detected by ELISA.

comparisons of the rank orders by Spearman's rank correlation test gave correlation coefficients of 0.789 between sites A and B, 0.796 between sites A and C, and 0.925 between sites B and C. Thus, the rankings were significantly correlated, implying that, allowing for differences in total disease incidence between sites, the varieties behaved similarly at each site.

Previous work (Xenophontos et al., 1998) suggests that several of the varieties used, including King Edward, Romano, Santé and Wilja, might have become systemically infected with M-type TRV. To detect such infections, progeny tubers from the field trials were grown on in the glasshouse and the emerging foliage tested by ELISA. However, for logistical reasons, it was possible to test only four progeny tubers from each plot. In these tests, systemic TRV infection was detected in Saxon, Shepody and Wilja from site A and in Romano from site C (Table 1).

Glasshouse tests

For comparison with the field trials, the same 15 varieties were subjected to glasshouse tests using soil from each of the three sites. However, the incidence of infection was very low when using soil from sites B or C. Daughter tubers on only one or two of the eight plants of the highly spraing-sensitive cvs Maris Bard and Pentland Dell developed symptoms, and no useful data were obtained. With soil from site A, the majority of Maris Bard and Pentland Dell daughter tubers developed symptoms. The overall symptom scores obtained using site A soil (Table 1) are in reasonable agreement with the combined scores from the field trials. The principal exception to this is Nadine, which was rather more severely affected in the glasshouse than at any of the field sites.

Leaves on plants growing from the daughter tubers were tested by ELISA. Systemic infections with M-type TRV were detected in plants grown from four of the eight groups of daughter tubers from Nadine, three of those from Wilja, and one each of those from Lady Balfour, Saxon and Shepody (Table 1).

Progress of disease

To test if the incidence of spraing symptoms in crops lifted early is less than in those that remain in the ground longer, randomly selected plants were harvested from plots of Pentland Dell and Santé at four different times. At the first harvest, skin set was incomplete; haulms were burnt down between the second and third harvests. The effect of storage after harvest, and the incidence of spraing in tubers of different size were also tested. The results are summarized in Table 2.

Table 2. Incidence of spraing symptoms (and % incidence) in tubers of Pentland Dell and Santé, larger (L) and smaller (S) than 45 mm, harvested at four dates and after different lengths of time in storage

Weeks in store	0		2		4		6	
Size class	L	S	L	S	L	S	L	S
Pentland Dell								
Harvest date								
7/8/02	0/19[1] (0%)	2/56 (4%)	1/19 (5%)	0/56 (0%)	2/19 (11%)	0/56 (0%)	3/19 (16%)	2/56 (4%)
21/8/02	2/19 (11%)	2/57 (4%)	3/19 (16%)	2/57 (4%)	0/19 (0%)	1/57 (2%)	1/19 (5%)	8/57 (14%)
4/9/02	3/24 (13%)	4/41 (10%)	2/24 (8%)	1/41 (2%)	4/24 (17%)	1/41 (2%)	2/24 (8%)	2/41 (5%)
18/9/02	3/22 (14%)	2/45 (4%)	5/22 (23%)	5/45 (11%)	4/22 (18%)	5/45 (11%)	2/22 (9%)	1/45 (2%)
Santé								
Harvest date								
7/8/02	2/49 (4%)	1/65 (2%)	2/49 (4%)	2/65 (3%)	3/49 (6%)	1/65 (2%)	3/49 (6%)	2/65 (3%)
21/8/02	5/69 (7%)	4/72 (6%)	4/69 (6%)	3/72 (4%)	3/69 (4%)	1/72 (1%)	4/69 (6%)	2/72 (3%)
4/9/02	2/63 (3%)	3/65 (5%)	3/63 (5%)	5/65 (8%)	6/63 (10%)	4/65 (6%)	4/63 (6%)	5/65 (8%)
18/9/02	7/64 (11%)	9/72 (13%)	8/64 (13%)	5/72 (7%)	5/64 (8%)	4/72 (6%)	8/64 (13%)	9/72 (13%)

[1] Number of tubers showing spraing symptoms/number of tubers examined.

The symptoms in Pentland Dell were mostly the classical necrotic arcs of spraing disease, whereas those in Santé consisted of more spotty, dispersed necrosis. To compare treatments, analysis of variance was applied to the percentage of symptom-bearing tubers in each of the samples (Table 3). Examination of the residuals from the analysis of variance showed that a transformation of these data was not necessary. Of the individual factors in the experiment, tuber size and harvest date had significant effects on spraing incidence (Table 3). The incidence in larger tubers (8.1% overall) was greater than that in smaller tubers (5.2% overall) (s.e.d. = 0.88%; $P = 0.04$). This effect was more evident in Pentland Dell (11.0% *versus* 4.8%) than in Santé (7.0% *versus* 5.5%), although the interaction between cultivar and size was not statistically significant (s.e.d. = 1.24%; $P = 0.09$). The total numbers of tubers with spraing symptoms showed an increasing trend with later harvest dates (3.4%, 5.2%, 6.6% and 10.1%, respectively) (s.e.d. = 1.24%; $P = 0.02$). However, after 0, 2, 4 or 6 weeks in store, the percentages of affected tubers were 6.4%, 6.4%, 5.5% and 7.2%, respectively, indicating that there was no significant increase during storage (s.e.d. = 1.45%).

Table 3. Analysis of variance of percentage data in Table 2

Source of variation	Degrees of freedom	Mean square	*F* probability
Harvest date	3	128.19	0.04
Cultivar	1	43.97	0.15
Harvest date × cultivar	3	0.88	
Tuber size	1	220.00	0.02
Harvest date × size	3	6.89	0.68
Cultivar × tuber size	1	76.33	0.09
Residual	3	12.26	
Time in store	3	4.17	0.86
Harvest date × time in store	9	19.66	0.35
Cultivar × time in store	3	3.01	0.91
Tuber size × time in store	3	12.35	0.54
Residual	30	16.74	

Tests on weeds and subsequent crop

In 2003, the year following the field trial, site A was sown with a commercial crop of spring barley, cv. Chalice. On 24 April, the day before the first herbicide application, a representative sample of the weed flora was collected. At this stage, the weeds were very small and unequivocal identification was difficult in a few cases. The roots of the weeds were tested for the presence of M-type TRV by inoculation of extracts to *C. amaranticolor* (Table 4).

On 17 June, 45 barley plants were collected randomly from the trial site. Extracts from the washed roots of groups of five plants were tested for the presence of TRV by RT-PCR, by ELISA and, after freezing and thawing, by inoculation to *C. amaranticolor*. Roots from glasshouse-grown barley plants served as a negative control in each

Table 4. Occurrence of M-type *Tobacco rattle virus* infectivity in roots of weeds collected from site A

Weed species	Number collected	Number infected
Chenopodium album	14	2
Galleopsis sp.	3	0
Matricaria discoidea (probably)	3	2
Senecio vulgaris	3	1
Stellaria media	11	10
Urtica urens	6	0

test. RT-PCR products specific for TRV were obtained from two samples, and one of these also gave a positive result in ELISA. No infectivity was recovered from any of the samples after freezing.

Discussion

The most familiar symptom produced in potato by infection with TRV is spraing (Cadman, 1959; Walkinshaw and Larson, 1959), which is mainly associated with primary infection. Stem-mottle (Rozendaal and van der Want, 1948) occurs in the haulm of some plants grown from spraing-affected seed, and is usually caused by infection with NM-type TRV isolates (Harrison and Robinson, 1982), although spraing-affected tubers may contain M-type or NM-type virus (Harrison et al., 1983). In some varieties, there may be no spraing symptoms, but fully systemic infection by M-type virus may be accompanied by discolouration of the edges or tips of leaflets and secondary growth and growth cracking of the daughter tubers (Xenophontos et al., 1998; Dale et al., 2000). Our observations on cv. Spey add another syndrome, comprising generalized mottle in leaves on all stems together with a virtually total failure to produce daughter tubers. Other plants in the same field were affected by apparently typical stem-mottle symptoms associated with NM-type infection, or by systemic M-type infection. It is likely that the NM-type virus was derived from the M-type virus by loss of RNA-2, and did not represent a distinct virus strain, but there is no straightforward way to verify this. Individual potato varieties are commonly prone to particular patterns of disease, but factors other than genotype are clearly also involved in determining the outcome of infection. One such factor might be the strain of TRV. However, comparison of the responses of 15 potato varieties at three TRV-affected sites provided no evidence of differences that could be attributed to differences among the virus isolates at those sites. In particular, there was no evidence that resistance broke down seriously at any site, or that highly spraing-sensitive varieties could escape being affected. Such differences as were observed were in the overall incidence of disease at the sites. Although several varieties produced spraing symptoms only at site A, the numbers of tubers affected were small relative to varieties such as Maris Bard and Pentland Dell, and no affected tubers were found when the overall incidence was lower, as at sites B and C.

Differences in overall incidence among the sites probably reflect differences in the numbers of viruliferous nematodes present and in their activity. Nematode activity in turn will have been affected by many environmental variables, especially soil water content and temperature (Cooper and Harrison, 1973; van Hoof, 1975, 1976). Differences in nematode activity over time probably also account for the observation that larger tubers were more often affected than smaller ones, different sized tubers probably being initiated at different times. Dale and Solomon (1988) reported that the glasshouse test they devised was more reliable than field trials, but that conclusion was not borne out in the experiments described here. The trials with the three soils were all done in the same glasshouse but, for practical reasons the tests with site A soil were started earlier in the year, when glasshouse temperatures were relatively cool, whereas those with soil from sites B and C were begun later, when temperatures were more difficult to control. Thus the conditions may not have been equally favourable for nematode activity in each case. In contrast to the glasshouse tests, the field trials at all three sites gave useful results, but it may be significant that conditions in 2002 were particularly favourable for nematode activity, with no shortage of rainfall or prolonged periods of extreme temperatures.

Taking field and glasshouse experiments together, systemic infection and spraing symptoms were both observed in several varieties, including Lady Balfour, Nadine, Romano, Saxon, Shepody and Wilja, sometimes in the same plant. This suggests that the outcome of infection of a TRV susceptible variety, i.e. whether the plant becomes systemically infected and/or develops spraing symptoms, is affected by environmental factors as well as by the genotype. Unlike the response to infection, resistance to infection in Record seems to be unaffected by environmental factors. No infection was recorded at any of the field sites, or in these or previous glasshouse tests (Xenophontos et al., 1998). Thus, Record seems to offer a promising source of durable resistance for the control of spraing disease.

Meanwhile, our results show that, with Record as the exception, almost any variety can be affected by spraing under certain conditions, including varieties such as Bintje, Hermes and Saturna that are usually regarded as resistant. Therefore, the only certain way to avoid spraing in a crop is not to grow potatoes on affected land. The disease progress experiment confirmed previous evidence (van Hoof, 1964; Harrison, 1968; Engsbro, 1973) that there is an increase in spraing incidence as harvest times become later. Importantly, this increase occurred only in tubers that remained in the ground, and not in tubers that were stored in the dark for an equal length of time. However, early harvest is probably not a practical way of avoiding the consequences of TRV infection. The results also suggest that using early test digs to assess the incidence of spraing may give misleadingly low estimates, as further symptoms may develop before harvest.

Our tests on the weed flora at site A confirmed the importance of weed hosts in maintaining TRV at a site. All the species found to be infected with TRV were ones that have previously been reported to be hosts for the virus (Cooper and Harrison, 1973). The plants that were sampled were young seedlings, not plants that had survived the winter. Of the species found to be infected, seed transmission has been reported only in *Senecio vulgaris* (Cooper and Harrison, 1973). Notably, extensive tests (Lister and Murant, 1967; Cooper and Harrison, 1973) failed to detect any transmission through the seed of *Stellaria media*, the species most frequently found to be infected in our tests. We conclude therefore that at least the majority of the weeds found to be infected at site A in April 2003 had been recently infected by viruliferous nematodes, implying considerable nematode activity very early in the growing season. Although TRV can be retained by viruliferous adult nematodes for months or years, it is not passed from mother to progeny (Ayala and Allen, 1968), nor is it retained through the moult. Thus, to maintain the virus at a site, each generation of nematodes must re-acquire it from plant roots, and infected weeds are the most likely potential source.

TRV infection was also detected in the roots of a small proportion of barley plants. Barley is generally considered to be a non-host for TRV; indeed, a barley crop has been shown to decrease the incidence of spraing in potatoes in the subsequent year (Maas, 1975). However, it is now clear that barley can be infected, but although M-type virus occurred in at least one of the plants tested, barley appears not to represent a significant reservoir of virus. Other cereal crops, including wheat and sweet corn, have also recently been reported to be hosts for TRV (Mojtahedi et al., 2002).

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