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Sustainable disease control using weeds as indicators: *Capsella bursa-pastoris* and Tobacco Rattle Virus

P P M IANNETTA*, G S BEGG*, T A VALENTINE* & J WISHART*†

*Environment Plant Interactions, Scottish Crop Research Institute, Invergowrie, Dundee, UK, and †School of Biology, St. Andrews University, St. Andrews, UK

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Summary

Arable weeds are believed to sustain disease outbreaks of the potato crop pathogen Tobacco Rattle Virus (*TRV*), which is particularly well-known for the costly damage it may cause to potato tubers. We describe a *TRV*-specific TaqMan™ based molecular-diagnostic quantitative RT-PCR method which showed that ecotypes of the widespread and common weed *Capsella bursa-pastoris* (shepherd's purse) are highly susceptible to *TRV* infection and may be suitable as indicator species of *TRV* presence *in situ*. Soils from two sites (S1 and S2), previously diagnosed as harbouring high levels of *TRV*, were the subjects of infection tests using *C. bursa-pastoris* and the susceptible model bait species *Petunia x hybrida*. *TRV* infection was only detected in all S1-soil, but in none of the plants grown in S2-soil. S1 soil had been treated annually with nematicide and herbicide, whilst continuing to cultivate *TRV* susceptible crops. S2 soil had been farmed for 5 years without the application of synthetic pesticides according to organic standards and had been sown with non-*TRV* susceptible crops in three out of the 5 years of the rotation. Our observations led us to question the current recommendations that: 'Weed control is important. Organic practices and set-aside may facilitate the re-introduction of *TRV* and/or the increase the distribution of the virus within a field'. We suggest that more effective and less environmentally damaging crop protection can be achieved using rotations that employ non-susceptible crops, in concert with management strategies that encourage crop-weed co-existence.

Keywords: Tobacco Rattle Virus, virus, nematode, qRT-PCR, shepherd's purse.

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Introduction

Tobacco Rattle Virus (*TRV*; genus *Tobravirus*) is a soil-borne plant pathogen that is transmitted between plants by the root-feeding ecto-parasitic nematode species *Trichodorus* and *Paratrichodorus*. *TRV* damages potato crops world wide causing serious loss of tuber quality as a result of spraing, which is obvious as discoloured arcs of necrotic tissue in the tuber flesh. *TRV* spreads slowly and is highly persistent, despite costly nematicide applications. Approximately 10 000 ha of UK farmland are treated annually with nematicides at a cost of *ca.* £2M, whilst crop losses due to *TRV* transmitted nematodes also cost about £2M (Dale & Neilson, 2006). Aldicarb is the principal nematicide for *TRV* control on conventional farms, though is allowable only if essential, and then only in certain European Union countries (Directive 91/414/EEC – Article 8). Aldicarb was withdrawn from use in potatoes and carrots in the UK in 2007, because it could not be applied at recommended rates without exceeding revised Maximum Residue Limits. In the absence of such pesticides to control nematode vectors (and broad-leaved weeds), control methods that meet integrated pest management (IPM)

Correspondence: P M M Iannetta, Environment Plant Interactions, Scottish Crop Research Institute, Invergowrie, Dundee DD25DA, UK.
Tel: (+44) 1382 560017; Fax: (+44) 1382 562426; E-mail: pietro.iannetta@scri.ac.uk or pete_iannetta@yahoo.co.uk

options have become more attractive; often these use low-input regimes that include organic production methods.

The roots of many common arable weeds species from infected fields have tested positive for *TRV* (Cooper & Harrison, 1973) and such data are extrapolated to suggest weed eradication as a general crop protection strategy, including *TRV* control (Dale & Neilson, 2006). The effectiveness of this strategy is arguable, as disease outbreaks are only ever suppressed and add to the already serious reductions of in-field wild plant diversity (Marshall *et al.*, 2003). Improved testing using weed species has been suggested to target (and so reduce) pesticide applications (Dale & Robinson, 2006; Evans, 2007).

An understanding of weed ecology at the intraspecific level is proposed for sustainable arable systems (Hawes *et al.*, 2005), for which *Capsella bursa-pastoris* L. Medic (shepherd's purse) is a useful model species (Iannetta *et al.*, 2007). Across arable farmed soils throughout the UK, *C. bursa-pastoris* is a common broad-leaved weed species [Farm Scale Evaluation (FSE) data: http://www.farmscale.org.uk/data.htm], and rapid-cycling [short time-to-flowering (TTF)] forms are the most common in-field type (Iannetta *et al.*, 2007). The relatively high abundance of *C. bursa-pastoris* may provide a large resource for nematode grazing and could pose an increased-risk to crop health. PCR diagnostics for definite identification of the *TRV* virus have been reported (Robinson, 1992; Kawchuk *et al.*, 1997; Mumford *et al.*, 2000; Holeva *et al.*, 2006). Here, we adapt the methods in a one-step TaqMan™ based quantitative RT-PCR, to quantify *TRV* in early- and late-TTF forms of *C. bursa-pastoris*. We test the hypothesis that in-field (time-to-flowering) variants of *C. bursa-pastoris* do not present an equal risk of *TRV* infection. We discuss our findings in the context of the current recommendations that include weed eradication for crop protection and that: 'Organic practices and set-aside may facilitate the re-introduction of *TRV* and/or the increase the distribution of the virus within a field' (Dale & Neilson, 2006; British Potato Council Report R276).

Materials and methods

Field soil sampling, weed and nematode diversity analysis

Soil was sampled in September 2006 from two locations, S1 (Dale & Neilson, 2006) and S2 (unpubl. SCRI obs.), previously confirmed to contain severe and persistent *TRV* infection. The crop rotations (from 2001) at S1 had been: potatoes, carrots, lettuce, spring barley, spring barley, potatoes. At S2 (managed organically since diagnosis), the rotation was: barley, oats, triticale and grass/clover mix (2 years). 10×1 kg of soil was collected randomly from a $4\ m \times 4\ m$ sample-grid, mixed and measured into pots for the glasshouse experiment. Weed seedbank diversity was assessed at each site using 3×1 kg of soil sieved into seed trays ($38\ cm \times 24\ cm \times 5\ cm$) and allowed to germinate.

Plant material and glasshouse experiment

Seeds of *TRV* (control) bait-plants (*Petunia x hybrida* Hort. Ex. Vilm) and four test *C. bursa-pastoris* ecotypes (Iannetta *et al.*, 2007; see Table 1), were surface sterilised and sown on sterile MS media in Petri dishes (20°C) for 7 days. Seedlings were transferred to 5 cm diameter pots of sterile compost for 21 days and then transferred into 12.5 cm diameter pots containing field soil or compost (negative control). Each replicate pot was placed inside a 15 L pot to protect it from splash contamination and positioned in a randomised six block-design with 1× replicate of either *Petunia* or *C. bursa-pastoris* on each field soil. Plants were grown for a further 6 weeks with fertigation [150 mL of 10% (wt/V) 20:20:20 Sangral soluble fertiliser (William Sinclair Horticulture, Lincoln, UK)] applied weekly and with additional routine watering when necessary. All pots were steam sterilised and plants grown at 25/15°C day/night regime. Ten replicates from each test type were harvested destructively, for drying and root weight measurements.

Table 1 Parental phenotypes showing a range of phenotypic characteristics and life-history traits

Accession	No. leaves	Days to flowering	Time-to-flowering	Fecundity (seed number)	Root mass (g^{-1} dwt)	% *TRV* incidence
156–233	25	56[a]	Early	209554[b]	0.396[a]	60
367–546	62	76[a]	Early	141307[b]	0.541[b]	100
798–188	88	112[b]	Late	80028[a]	0.547[b]	60
937–71	100	100[b]	Late	109524[a]	0.721[c]	100

Values followed by the same superscript letter denote data that is not significantly different (at $P < 0.05$).

Molecular diagnostics for nematode vectors and TRV

After harvesting, roots and leaves were washed and frozen in liquid nitrogen. Ten 300 g of samples were used for nematode (Brown & Boag, 1988) and DNA extractions (Donn *et al*., 2008) for PCR using specific primers (Boutsika *et al*., 2004; for *TRV* vector nematodes). DNased root RNA (100 mg; RNeasy™, Qiagen, Crawley, UK) was tested for *TRV* in a one-step quantitative RT-PCR [adapted from Mumford *et al*., 2000; Applied Biosystems™ (Carlsbad, CA, USA) 7500 Fast Real-Time PCR system with TaqMan Fast Universal PCR MasterMix], with *M-MLV* reverse transcriptase (RNase H Minus # M5301; Promega™, Madison, WI, USA), with optimal concentrations of primers (300 nM) and probe (150 nM). Reactions were carried out in duplicate and repeated (from extraction) to ensure reproducibility. *TRV* estimates were normalised against the C_t-value for ACTIN-2 [primers used at 300 nM (Hisamatsu *et al*., 2005)], from the same RNA in an RT-PCR using SYBR green (MESA GREEN qPCR MasterMix Plus for SYBR Assay I Low ROX; Eurogentec™, Southampton, UK). Only samples that produced a signal for actin were included in any analyses.

Statistical analysis

ANOVA and Poisson regression were used to test differences between sites in weed density and species number, and between the proportion of *TRV positive* plant ecotypes and compost grown controls. The significance of the treatment effects was determined by conditional *F*-test in the case of ANOVA and log-likelihood ratio tests for Poisson regression.

Results and discussion

The species-specific diagnostic PCR (Boutsika *et al*., 2004) confirmed the presence of the *TRV* virus vector nematodes (*Trichodorus*) at both sites. However, no *TRV* infection was found in plants cultivated on soil from S2 (data not shown). All *Petunia* and *C. bursa-pastoris* replicates grown on soil from S1 showed *TRV* infection. One hundred percent incidence of *TRV* was recorded in RNA from *C. bursa-pastoris* ecotypes 367–546 and 937–71 (Likelihood ratio test: d.f. = 4, deviance = 9.085, $P = 0.05$), as opposed to 60% (three of five replicates), for accessions 156–233 and 798–188. We concluded that while *C. bursa-pastoris* TTF variants tested were all susceptible to *TRV* infection, they were not equally so and we therefore accepted our hypothesis.

Though no correlation was found between TTF and *TRV* susceptibility, early TTF forms may pose an increased-risk to crop health, as they are most common in arable fields (Iannetta *et al*., 2007), and have high fecundity. Where *TRV* infection is systemic (as for 367–546), in early TTF forms it is also transferable to seed (Cooper and Harrison's study 1973). The possibility that *C. bursa-pastoris* ecotypes with more extensive root systems are more susceptible to *TRV* was not supported (Table 1). The high *TRV*-susceptibility of *C. bursa-pastoris* matches that of *Petunia*, but *C. bursa-pastoris* may also be used as an *in situ* indicator of *TRV* presence and disease-risk.

The absence of *TRV* at S2 was surprising, given the severity of *TRV* outbreaks and tests before 2001 and before the ground converted to organic production. At S2, the single control measure involved a rotation of non-*TRV* susceptible cereal crops, plus a 2-year grass-clover ley. In contrast, *TRV* presence at S1 was confirmed as continuous, despite the soil having been treated regularly with nematicides and herbicides. At S1, three *TRV* susceptible crops had been cultivated within the previous 6 years (two potatoes and one carrot). Weed relative abundance did not relate to *TRV* infection rates, as weed seedbank levels were greatest at S2 (Table 2). Although *C. bursa-pastoris* was not recorded at either site, other *TRV* weed biomonitors, *Stellaria media* L. (common chickweed) and *Viola arvensis* L. (field pansy) were found (Cooper & Harrison, 1973), the former at both sites and the latter at only S2. *TRV* presence did not therefore correlate with the presence of known susceptible weeds. Cooper and Harrison (1973) reported increased crop *TRV* infection rate for potato grown on herbicide treated weed-free sites, possibly because there were no alterative food sources. Increasing within-field weed functional diversity alone can present a lower risk to crop health (Berenbaum & Zangerl, 2006). In addition, the persistence of *TRV* at S1 (a Links soil), does not correlate with its designation from a comprehensive survey of *TRV* in relation to soil type (Cooper, 1971) as representing a lower risk than the Boyndie soil found at S2. The data suggests that the risk posed by

Table 2 Weed soil seedbank diversity measures (averages; of $n = 3$) recorded from sites S1 and S2, giving the mean values (across replicates) of weed species and individuals found at each location

	Site (S) average (kg^{-1} soil)		
Diversity	S1	S2	*P*-value
Individuals	78	120	0.026
Species	7	10	0.024

A significance test result for mean data is denoted by the '*P*-value'.

weeds is lower than the risk posed by the cultivation of a susceptible crop in the rotation, such as potatoes.

The application of non-specific nematicide kills all nematode groups (Sturz & Kimpinski, 1999) and other ecologically important soil microorganisms, such as rhizosphere bacteria that promote crop growth (Sturz & Kimpinski, 1999; O'Flaherty *et al*., 2003). Similarly, broad-spectrum herbicides to control *TRV* have non-specific effects that will impact negatively upon arthropod diversity and on the food-web (Moreby & Southway, 1999). However, weed eradication is still presented as a main management option, despite scientific evidence showing that effective *TRV* management may be achieved using low-input crop rotations that employ non-*TRV* susceptible monocotyledonous crops. Based on these studies, we suggest that low-input rotations should be examined as the default means of disease control for *TRV* and other pathogens, and exercised in conjunction with monitoring using molecular diagnostics of in-field weeds to assess disease risk, ahead of re-cultivation with susceptible crops.

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