IDENTIFICATION OF VIRUSES IN PROCESS TOMATOES USING COMMERCIAL ELISA KITS

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SUMMARY

Commercial ELISA test kits were used to test crop and weed samples from process tomato crops in Poverty Bay and Hawke's Bay for incidence of eight virus diseases. Tomato spotted wilt virus (TSWV) was the most frequently identified virus in tomatoes and was present in six common weed species. Alfalfa mosaic and cucumber mosaic (AMV and CMV) were the next most common. Other viruses identified were tomato aspermy (TAV), potato X (PVX), potato Y (PVY), tomato mosaic (TmMV) and tobacco mosaic (TbMV). Tomato yellow top (TYTV), was detected in tomato plants using non-commercial ELISA. Incidence of TSWV was visually estimated at 1-2% in most fields (maximum 20%). Estimated incidence of other viruses did not exceed 1%.

Keywords: ELISA, tomatoes, virus identification, *Lycopersicum esculentum*, weed hosts

INTRODUCTION

In the 1990/91 season, 37,200 t of process tomatoes (*Lycopersicum esculentum*) were produced on approximately 556 ha in Hawke's Bay (average 67 t/ha) and 52,500 t on 710 ha in Poverty Bay (average 74 t/ha). Of the various factors that can affect yields, virus diseases have been one of the most difficult to assess due to the wide and variable range of symptoms. The traditional methods of identifying viruses, by inoculation to indicator plants has been slow and costly, resulting in a lack of information on the viruses present in process tomatoes.

In the 1987-88 and 1988-89 seasons, crop monitoring in Hawke's Bay (A.J. Kale, unpublished) identified serious virus problems, probably caused by TSWV, in certain tomato fields (estimated 60% plants infected). In the 1989-90 season CMV was prevalent (identification based on field symptoms and limited indicator-plant tests) with an estimated average of 34% of plants infected (K.G. Tate and D.W. Manktelow, unpublished). By contrast, in that season, TSWV averaged only 8% incidence and AMV 3%.

Virus detection kits, based on enzyme-linked immunosorbent assay (ELISA) techniques, have become commercially available for a range of plant viruses. The potential use of these for rapid and economical indexing of large numbers of plant samples and for training field personnel in visual identification of virus disease was investigated.

METHOD

Nine ELISA test kits for eight viruses of tomatoes previously recorded in New Zealand (Pennycook 1989) were obtained from Agdia Inc, 30380 County Rd 6, Elkhart, IN46514, USA. The nine kits tested separately for the presence of AMV, CMV, TSWV-L (Lettuce isolate), TSWV-I (Impatiens isolate), TbMV, TmMV, TAV, PVX and PVY.

Tomato fields at various stages of growth were visited and single plants showing virus symptoms photographed, the symptoms recorded, viruses tentatively identified

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for plants with distinctive symptoms and foliage samples taken for laboratory analysis. A total of 184 tomato plants were tested during December and January, 1990-91, from a total of 29 fields in Hawke's Bay and Poverty Bay.

Samples were processed as soon as possible after collection, according to the test kit instructions. Extracted sap was stored at 5-8 °C overnight and ELISA-tested the next day, with results being recorded by late afternoon. Each ELISA run included one positive control (supplied by Agdia Inc) and one negative control for each virus, and sap from up to 50 plant samples. Microwell colour change was read visually, without the aid of a microplate reader. Surplus sample material was stored at 4-6 °C and, if the ELISA tests gave unclear or unusual results, was sent for confirmation tests at DSIR Plant Protection, Lincoln. Fifty samples were retested in this way, using non-commercial ELISA, sap inoculation to herbaceous indicator-plants and electron microscopy. This enabled TYTV (a strain of potato leaf roll virus), for which a commercial ELISA Kit was not used, to be identified.

After each set of samples was processed, results were compared with initial field notes and photographs. Diagnostic symptoms were identified so that confidence in field diagnosis of viruses rapidly improved over the survey period.

Virus incidence in each field was estimated on the basis of symptoms associated with each virus. These estimates, subjective assessments of overall incidence, were made by the authors during field visits. Weeds are acknowledged hosts of many plant viruses and in February 1991, 82 weed samples from crop headlands and adjacent roadsides were similarly tested by ELISA. Each sample contained 4-5 well spaced plants with or without virus disease symptoms.

RESULTS AND DISCUSSION

Viruses identified and symptoms expressed in tomatoes

In both Hawke's Bay and Poverty Bay TSWV was frequently identified (in 51% of samples), followed by AMV (24%) and CMV (19%) (Table 1). Presence of other viruses detected was at much lower levels, namely PVX 15%, TAV 10%, TYTV 4%, PVY 3%, TmMV 0.5% and TbMV 0.5%. Since only visibly diseased plants were sampled the above values do not represent actual virus frequencies in the crops. TSWV incidence was estimated at 1-2% in 17 fields, 2-10% in 11 and 20% in the remaining field. There were no apparent differences in the range of TSWV incidence between the two districts. The other viruses were conservatively estimated at frequencies of up to 1%.

TABLE 1: Numbers and percentage of tomato plant samples testing positive¹ for viruses in the 1990-91 season.

Virus	Hawke's Bay	Poverty Bay	Mean %
TSWV-L	67	26	50.5
TSWV-I	0	0	0.0
AMV	26	18	23.9
CMV	24	11	19.0
PVX	24	3	14.7
TAV	11	8	10.3
TYTV ²	4	3	3.8
PVY	6	0	3.3
TmMV	0	1	0.5
TbMV	1	0	0.5
Mixture ³	45	14	32.1
Total samples	105	79	

¹ This does not represent virus frequency in the crops as symptomless plants were not tested.

² Of 62 samples tested at DSIR, Lincoln only

³ Up of 4 viruses detected in one plant

The above estimates of field incidence were visual, symptom-based assessments. The presence of latent virus infections where symptoms were mild or absent, or multiple infections where symptoms of one virus masked the others, meant that accurate field assessment of virus frequency based on symptoms alone was not possible. Nevertheless, if crop monitoring personnel could confidently identify viruses in plants exhibiting distinctive symptoms, they may detect virus problems in time to assess possible effects on yield, perhaps reduce disease spread, and focus attention on any unusual symptoms. This would assist growers to be pro-active in virus control the next season. TSWV initially exhibited a confusing array of symptoms in Hawke's Bay, but after repeated ELISA testing a pattern of diagnostic symptoms could be summarised (Table 2). By contrast, in Poverty Bay, field identification of TSWV was comparatively easy because characteristic symptoms, such as bronzing, were usually present. CMV and AMV usually exhibited characteristic symptoms and were readily identified. However, mild infections of CMV were not easily identified. No clear diagnostic symptoms were determined for the other viruses.

Local observations (I.S. Brice, unpublished) and similar observations overseas (Yudin *et al* 1986), suggest that TSWV can have devastating effects on tomato yields. This survey was not designed to assess crop losses, but experience with the ELISA kits indicate that they have a useful role in identifying viruses in surveys of losses caused by these diseases.

TABLE 2: Diagnostic symptoms for TSWV, CMV and AMV in process tomatoes, Hawke's Bay and Poverty Bay.

TSWV	Leaflets	Purple veins, uprolling $\pm$ 'bronzing to necrotic
	Petioles	Downcurled, $\pm$ dark streaks
	Stems	$\pm$ dark streaks
	Fruit	$\pm$ ringspots/surface distortion
	Whole plant	Stunted/bunched, short internodes
	Single laterals	Abnormally erect, bunched, purple tinge, $\pm$ bronzing
CMV	Leaflets	Narrow/spikey, "little" leaves, to "shoestring" deformation
	Whole plant	"Open" foliage to "thistle-like" appearance. Not stunted
AMV	Leaflets	Bright yellow or creamy/chlorotic mottle
	Fruit	$\pm$ greasy brown variable markings, $\pm$ surface distortion
	Whole plant	Normal habit, flecked with yellow

'may or may not be present

Alternate virus hosts and potential inoculum sources

Six common weeds each tested positive for TSWV, CMV and AMV, three each for PVY and TmMV and two each for TAV, TbMV and PVX. Thorn apple (*Datura stramonium*), amaranth (*Amaranthus* spp.), black nightshade (*Solanum nigrum*), fathen (*Chenopodium album*) and Jerusalem cherry (*Solanum diflorum*), common in and around tomato fields, were the main carriers of TSWV (Table 3). Similar reservoirs of infection for TSWV were reported by Cho *et al* (1986) in Hawaii.

Several of these weeds are also known hosts of the western flower thrips (*Frankliniella occidentalis*) a vector of TSWV (Yudin *et al* 1986). This may help to account for the occasional severe outbreaks of TSWV that have occurred in Hawke's Bay and Poverty Bay. Further monitoring of TSWV in tomatoes, weeds and thrips may help to formulate strategies for reducing TSWV levels in process tomato crops. Similarly, aphids are known vectors for CMV, AMV and TAV, so the presence of these viruses in common weeds associated with tomatoes provides a reservoir of infection for disease outbreaks in years favourable for aphid activity, such as in 1989-90.

TABLE 3: Numbers of weed samples¹ testing positive for viruses in Hawke's Bay and Poverty Bay during February 1991.

Weed species	Total samples	AMV	CMV	TSWV-L	PVY	PVX	TmMV	TbMV	TAV
amaranth (<i>Amaranthus</i> spp.)	9	3	6	4 ²		1	1	1	
Bathurst burr (<i>Xanthium spinosum</i>)	1								1
black nightshade (<i>Solanum nigrum</i>)	9	3	1	3					
broad leaved plantain (<i>Plantago major</i>)	1								
Californian thistle (<i>Cirsium arvense</i>)	5								
chamomile (<i>Anthemis nobilis</i>)	1								
dock (<i>Rumex</i> spp.)	4		1					1	
fathen (<i>Chenopodium album</i>)	7	3	1	2 ²		1			
fennel (<i>Foeniculum vulgare</i>)	1								
inkweed (<i>Phytolacca octandra</i>)	3								
Jerusalem cherry (<i>Solanum diflorum</i>)	2	1		2					
mallow (<i>Malva</i> spp.)	6						1		
perennial sow thistle (<i>Sonchus arvensis</i>)	7		1	1	1				
portulaca (<i>Portulaca oleraceae</i>)	1								
prickly sow thistle (<i>Sonchus asper</i>)	1	1							
shepherd's purse (<i>Capsella bursa-pastoris</i>)	1				1				
thorn apple (<i>Datura stramonium</i>)	5			5	2		1		
white clover (<i>Trifolium repens</i>)	5	3	3						1
wild turnip (<i>Brassica rapa</i> ssp <i>sylvestris</i>)	1								
yarrow (<i>Achillea millefolium</i>)	2								

¹Samples contained foliage from 4-5 well-spaced plants²Included 1-2 samples positive for Impatiens isolate (TSWV-I)**Comments on ELISA**

ELISA is well suited for surveying field crops for specific viruses (Thomas 1988) and has previously been used in New Zealand to survey virus diseases in potatoes, peas, dwarf beans, broad beans and lentils (Fletcher unpublished). The commercial kits are likely to expand the use of ELISA in New Zealand for routine identification of plant viruses. While the kits were designed to efficiently test large sample numbers, microplates were easily divided so that any number could be tested at once. In this study, sets of up to 50 samples were found to be manageable and economical on reagents, with results available within 30 hours of sampling. Each set of samples tested required a minimum of two (but preferably replicated) microwells for positive and negative control samples, so larger sets of samples provided most efficient use of the kits. Confirmation tests are recommended, using indicator plants, electron microscopy and serology. In this survey 50 of the 257 samples were further tested to confirm initial diagnoses. Some diagnostic discrepancies did occur, but these could be attributed to different sub-samples being used, lack of replication of controls and the greater sensitivity of some indicator plants to low virus titres compared with the kits. The use of a microplate-reader, though not essential would be necessary to provide quantitative data and improve precision in reading results for slow or weakly reacting tests (eg CMV).

Commercial ELISA kits for plant virus detection offer the potential for routine, economical indexing of large numbers of samples. When confronted with symptoms not pertaining to specific viruses, the rapid diagnosis permitted by the ELISA kits allows potential virus problems to be identified by field staff at an early stage, and may make it possible for more active in-season disease management than has been attempted in the past. Thus there is potential to include ELISA virus indexing as part of a comprehensive, integrated pest and disease management plan for process tomato crops.

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