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Buttercup squash virus diseases
—research report 1996/97

A report prepared for the
New Zealand Buttercup Squash Council

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1 EXECUTIVE SUMMARY

In 1996/97 we continued our programme of research on squash virus disease control. We surveyed crops nationally and only found watermelon mosaic virus (WMV) in Hawke's Bay. Once again, zucchini yellow mosaic virus (ZYMV) was not detected in squash crops, however some weeds being infected. In Hawke's Bay, only mid and late sown crops developed any disease, the levels were below 5%, apart from one case of 40% WMV. We continued to find virus overwintering in weeds. We collected aphid flight data to provide a base of information about virus vectors' flight patterns. We also noted the presence of two further potential aphid virus vectors.

Using field trials we further confirmed the potential yield loss in three squash types, Delica, Arjehei and Miyako, from mid-season virus infection. In particular, ZYMV and mixed virus infections increased the incidence of non-marketable fruit by reducing overall size and weight and affecting fruit appearance. WMV caused no significant yield losses apart from some fruit blistering. Miyako had more non-marketable fruit than the other two types. We further confirmed seed transmission (1-2%) of both viruses from infected fruits in laboratory experiments.

We will continue to work on the identification and study of alternative hosts, epidemiology, vectors, and seed transmission. Emphasis will be on confirming aphid flight patterns. Work will commence on determining action thresholds to control aphid infestation within squash crops. Information gathered will assist growers to make sensible management decisions about disease risk, time of sowing, and chemical management of squash crops.

2 INTRODUCTION

This report summarises research on virus disease management funded both by the Crown (PGSF 96 CRF-04-4937 Diseases of arable and vegetable crops) and the New Zealand Buttercup Squash Council during 1996/97. The work is a continuation of that commenced in 1995 (Fletcher & Jermyn) and furthered in 1996 (Fletcher 1996). Research this season covered disease epidemiology, aphid flight patterns, and disease loss effects. It exemplifies the integral collaboration necessary between research and industry to study and resolve the problem of virus management in buttercup squash.

3 METHODS

3.1 National squash virus disease survey

A national survey of squash crops representing the main regions was undertaken from December 1996/97 through to February 1996/97. In consultation with Squash Council National Delegates contact was made with growers and samples of squash crops were surveyed. As in 1995/96, eight regions were covered: Northland (Dargaville), Auckland (Pukekohe, Mangatangi), Bay of Plenty (Te Kaha), East Coast (Gisborne), Hawke's Bay (Hastings), Manawatu (Palmerston North), Marlborough (Blenheim) and Canterbury (Ashburton, Rakaia).

3.2 Disease epidemiology

3.2.1 Weed survey

Overwintering weeds in and around the sites of Hawke's Bay squash crops in which disease had been noted the previous season were collected and assayed for possible virus infection. A site with squash and a number of other vegetable crops was also monitored for virus disease development.

3.2.2 Squash volunteers

Volunteer squash seedlings were collected from two sites where infections were noted in the previous season and assayed for possible virus infection.

3.2.3 Site monitoring

A block in Pakohai Rd, Hawke's Bay, regularly cropped with squash, was monitored during the season for virus disease development. Virus infection in weeds and crops were recorded in order to detect any infection pattern.

3.2.4 Seed transmission

Seeds from fruit infected with a virus from the varietal yield trial were sown, grown and assayed for seed-borne watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) transmission.

3.2.5 Aphid vectors

Aphid flight patterns were monitored at Lawn Rd, Hastings, from September 1996 through to May 1997. A wind trap to trap aphids, set at 2.5 m, was emptied weekly and the aphids counted and identified.

3.3 Squash virus yield effects

To further understand the potential effects of WMV and ZYMV on squash crop yields a replicated yield trial was again established at Lawn Road, Hastings. The objective this season was to compare three major squash types (Delica, Arjehei, Miyako) and their response to a mid-season virus infection. Experimental treatments included: single WMV or ZYMV infection, mixed virus infection and control. The experimental plots of Delica, Arjehei, and Miyako were sown on 1 November 1996 at 40 cm spacings, 10 plants/plot, 1.5 m wide rows with five replications. The trial was managed according to recommended squash growing guidelines (King & Wishart 1993). The trial was inoculated by hand on 3 January 1997. The plots were hand weeded, and plants trained for easy assessment and harvest. The crop was sprayed three times for powdery mildew. The trial was harvested, weighed and assessed for quality on 6 March 1997, 126 days after sowing.

4 RESULTS

4.1 National squash virus disease survey

In total, 155 crops of squash, zucchini, melon and other cucurbits were surveyed. No sign of either WMV or ZYMV was recorded in most regions. Only in Hawke's Bay did WMV disease develop during the season. Once again, as in 1995/96, no ZYMV was detected in any surveyed crop.

4.2 Hawke's Bay squash virus monitoring

Results are summarised in Figure 1a along with a 1995/96 comparison (Fig. 1b). Early sown crops developed only trace levels of WMV. Mid-sown crops developed disease slowly with trace levels evident and one crop with 2-5% incidence. Late sown crops were similar to mid-sown, developing only trace, 5% or in one case up to 40% WMV.

4.3 Disease epidemiology

4.3.1 Weed surveys

A number of weed species were found to be infected with WMV, and sometimes ZYMV (Table 1). Some species were quite widespread early in the spring (wild carrot, speedwell, stagger weed, cleavers and red root) both around crop margins and as seedlings within a previous season's crop. Additional virus hosts to those of previous surveys included broad-leaved dock, fathen, sow thistle, twin cress, vetch and white clover. Aphid species associated with some of these weeds included the established cucurbit virus vectors: *Myzus persicae, Aphis craccivora, Aphis gossypii, Macrosiphum euphorbiae, Acythosiphon pisum* and *A. kondoi.* In addition, two recently recognised cucurbit virus vectors, *Hyperomyzus lactucae* and *Lipaphis erysimi,* were found.

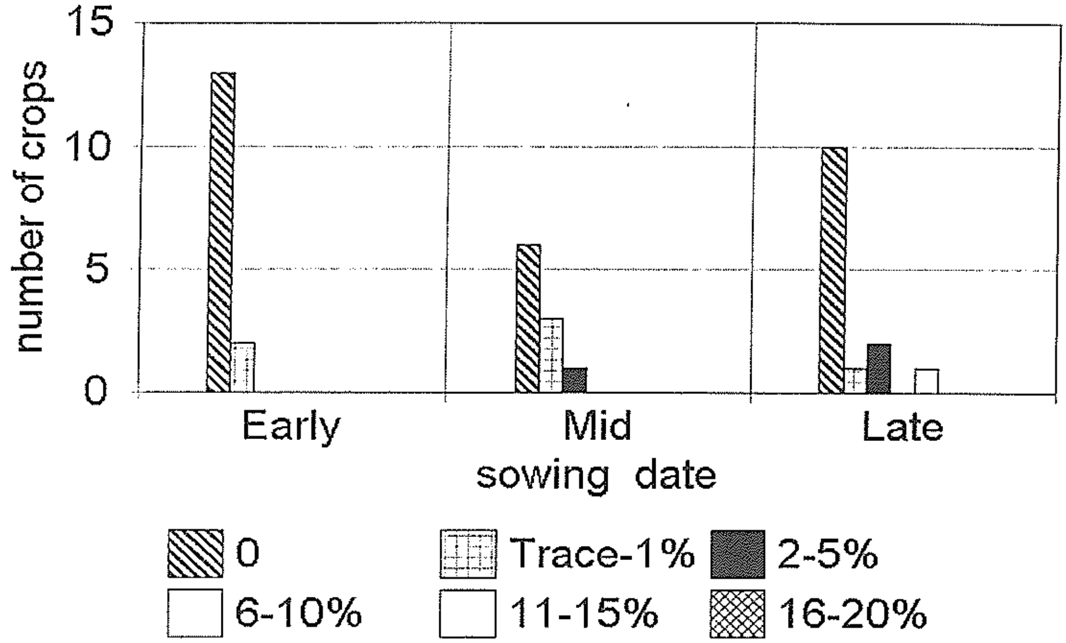

Figure 1a: **Development of WMV infection in buttercup squash in Hawke's Bay 1996/97.**

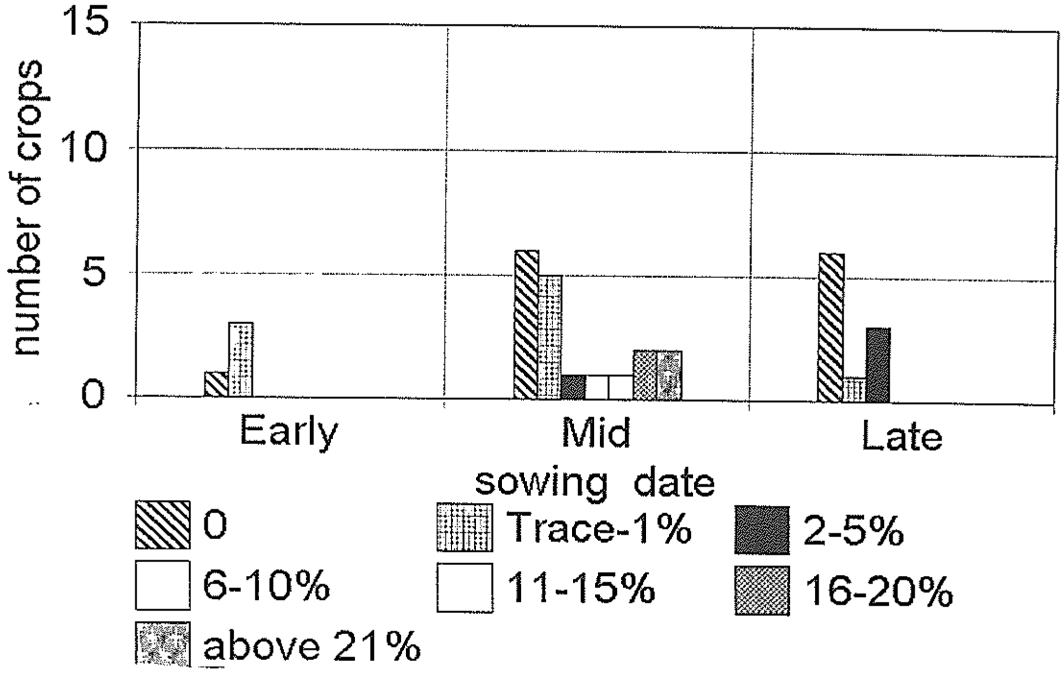

Figure 1b: **Development of WMV infection in butercup squash in Hawke's Bay 1995/96.**

Table 1: WMV and ZYMV and associated aphids in weeds in and around squash crops Central Hawke's Bay, 1996/97.

Weeds	Virus	Associated aphids	
Black nightshade	*Solanum nigrum*	WMV	
[1]Broad-leaved dock	*Rumex obtusifolius*	WMV	
Broad-leaved plantain	*Plantago major*	WMV	
Chickweed	*Stellaria media*	WMV, ZYMV	
Cleavers	*Galium aparine*	WMV, ZYMV	*M. euphorbiae*, *A. pisum*, *A. kondoi*, *A. gossypii*
[1]Fathen	*Chenopodium album*	WMV, ZYMV	*M. euphorbiae*, *M. persicae*
Fennel	*Foeniculum vulgare*	WMV	
Field madder	*Sheradia arvensis*	WMV	
Hawks beard	*Crepis* sp.	WMV	
Hemlock	*Conium maculatum*	WMV	
Horehound	*Marrubium vulgare*	WMV	
Oxtongue	*Picris echioides*	WMV	*M. euphorbiae*,
Rayless chamomile	*Matricaria dioscoidea*	WMV	
Redroot	*Amaranthus* sp.	WMV	
[1]Sow thistle	*Sonchus oleraceus*	WMV	*H. lactucae*, *M. rosae*
Speedwell	*Veronica* sp.	WMV	
Stagger weed	*Stachys arvensis*	WMV, ZYMV	
[1]Twin cress	*Cheiranthus didymus*	WMV	*B. brassicae*, *A. kondoi*, *L. erysimi*
[1]Vetch	*Vicia sativa*	WMV	*A. gossypii*
[1]White clover	*Trifolium repens*	WMV	
Wild carrot	*Daucus carota*	WMV	

[1] Additional hosts first recorded 1996/97.

4.3.2 *Squash volunteers*

WMV was detected in 27/30 volunteers, ZYMV in 3/30 and CMV in 3/15 volunteer squash plants assayed.

4.3.3 *Site monitoring*

Figure 2 illustrates the progression of disease from virus-infected weeds within the crop, and from weeds on the roadside fence line. During the previous season (1995/96) squash in block 2 was 5-10% infected with WMV. No WMV-susceptible winter crop was grown following this squash crop, so carry-over was most likely through weeds. This was confirmed in October 1996 when surveys of weeds in the rotation blocks detected virus in chickweed and rayless chamomile, in association with likely aphid vectors. Along the weedy fence line WMV was found in cleavers, twin cress, white clover, vetch, chickweed and rayless chamomile. Virus was further detected in sow thistle and ox tongue during the season.

In January 1997 WMV began to appear in block 3, most likely from infected chickweed and rayless chamomile in block 2. In March, infection was first observed in runners from block 1 as they made contact with the weedy roadside fence line.

4.3.4 *Seed transmission*

Assays of seed collected from fruits infected with virus from the yield trial showed transmission of 1-2% of ZYMV and WMV.

4.3.5 *Aphid vectors*

Wind trap catches of aphids at Lawn Road are summarised in Figure 3a. Highest total aphid flight numbers were recorded in a spring flight peak in late October 1996. Virus vectors (*Myzus persicae, A. craccivora, A. gossypii, Macrosiphum euphorbiae, Cavariella aegopodii,* and *Rhopalosiphum padi*) peaked in late September (Fig. 3b). Autumn peak flights were in early March 1997 but once again vectors peaked a little earlier. Vector activity diminished over the warmer January/February months in line with overall aphid activity.

Figure 2: **WMV infection pattern in Pakohai Rd, Hawke's Bay, squash and pumpkin crops 1996/97.**

Rotation	**Block 1**	**Block 2**	**Block 3**
1995/96 (Nov-Mar)	onion	squash (5-10% WMV)	spinach
(Mar-Nov)	brassica	potato	potato
		*	
		*	++++++++ +++
			+++++ +++
		* *	* +++ *
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1996/97 (Nov-Mar)	squash + + + + + + + ++	brassica	pumpkin
	+++++++++++		
	weedy fence line * * * + +	+ * * + *	*
	main road		

Key

WMV disease development in January 1997 ********

in March 1997 ++++++++

Summary of observations

October 1996 WMV found in cleavers, vetch, white clover, rayless chamomile, twin cress, and chickweed, along fence line and in old squash crop.

Aphids present: *M. euphorbiae, A. kondoi, A. pisum, H. lactucae*

November 1996 WMV found in sow thistle and ox tongue.

March 1997 WMV found in ox tongue.

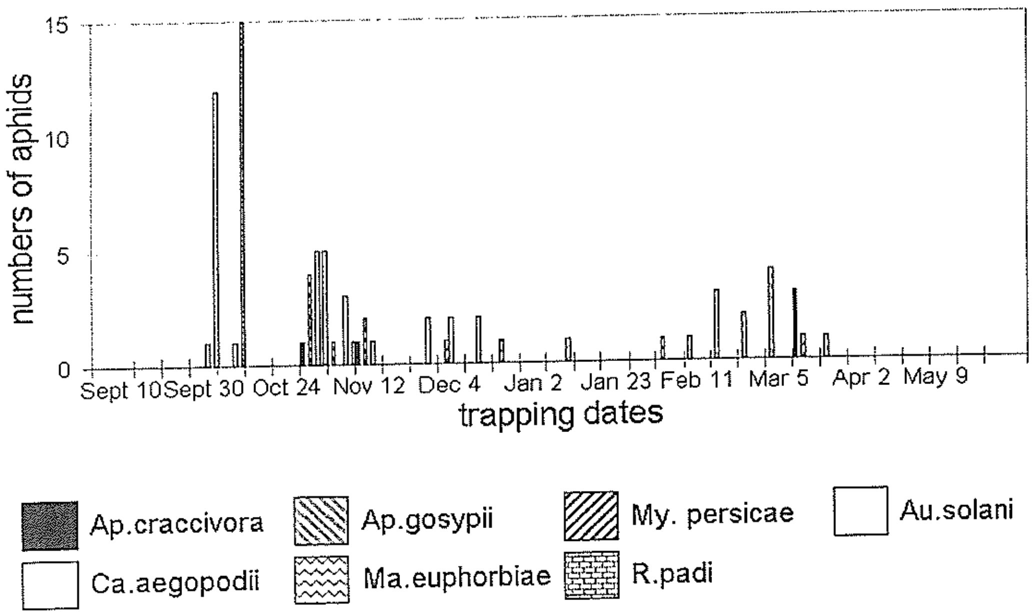

Figure 3a: ZYMV and WMV aphid vectors recorded at Lawn Road, Hawke's Bay, 1996/97.

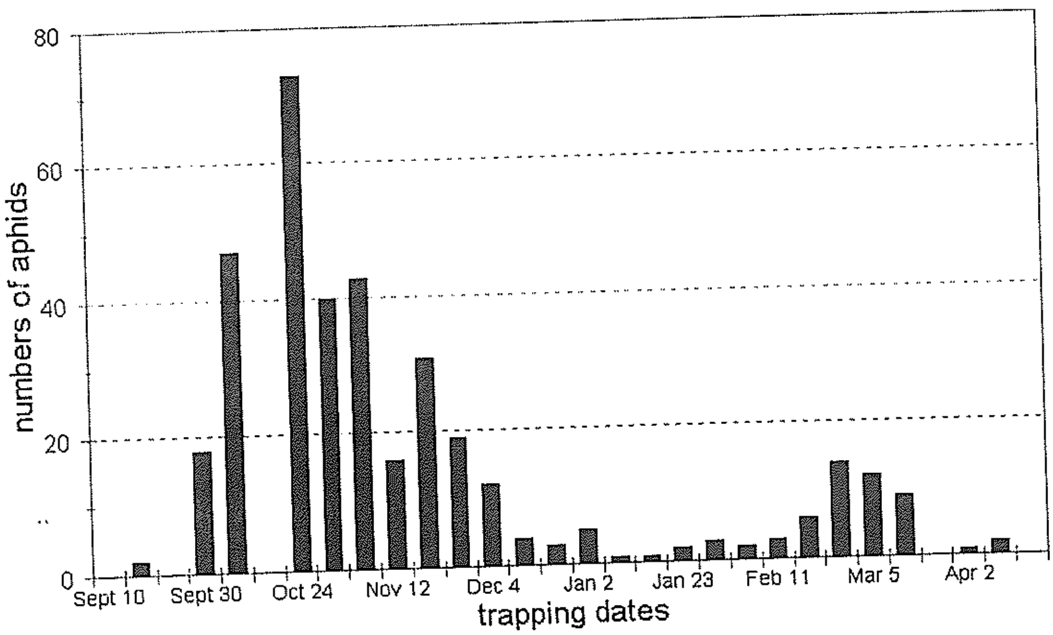

Figure 3b: Aphids trapped in buttercup squash at Lawn Road, Hawke's Bay, 1996/97.

4.4 Squash virus yield effects

The effects of mid-season virus inoculation on the yield and marketability of Delica, Arjehei and Miyako are summarised in Tables 2-7.

Table 2: Mean total weight of squash (kg/plot) and percentage reduction relative to control from three cultivars inoculated with ZYMV and WMV.

	Cultivars						Treatment mean	
Treatment	Delica	%	Arjehei	%	Miyako	%		%
Control	30.9[1]		28.6		23.1		27.5	
ZYMV	24.0	22	19.4	32	16.2	30	19.9	28
WMV	28.8	7	25.6	10	22.2	4.0	25.5	7
WMV + ZYMV	20.4	34	19.2	33	17.2	26	19.0	31
Cultivar mean	26.0		23.2		19.7			
LSD (5%)	3.03[2]						7.38[3]	

[1] For comparing treatments for a given cultivar, LSD (5%) = 8.59.
[2] LSD (5%) for comparing cultivar means.
[3] LSD(5%) for comparing treatment means.

Mean total weight was significantly reduced by ZYMV and mixed virus infection by 28 and 31% respectively (Table 2). WMV did not significantly reduce total weight.

Table 3: Mean weight of marketable fruit (kg/plot) and percentage reduction relative to control from three cultivars inoculated with ZYMV and WMV.

	Cultivars						Treatment mean	
Treatment	Delica	%	Arjehei	%	Miyako	%		%
Control	19.3[1]		19.5		11.6		16.8	
ZYMV	9.9	48	8.4	57	2.3	80	6.9	59
WMV	18.3	45	16.1	18	7.1	39	13.8	18
WMV + ZYMV	9.3	52	8.6	56	3.0	74	7.0	59
Cultivar mean	14.2		13.1		6.0			
LSD (5%)	3.16[2]						5.13[3]	

[1] For comparing treatments for a given cultivar, LSD (5%) = 7.45.
[2] LSD (5%) for comparing cultivar means.
[3] LSD (5%) for comparing treatment means.

Mean marketable weight/plot was significantly reduced by ZYMV and mixed virus infections by 59% (Table 3). WMV did not significantly reduce weight of marketable fruit (Table 3). Miyako had a significantly lower weight than the other cultivars (Table 3).

Table 4: Mean number of marketable squash (fruit/plot) and percentage reduction in yield relative to control from three cultivars inoculated with ZYMV and WMV.

	Cultivars						Treatment mean	
Treatment	Delica	%	Arjehei	%	Miyako	%		%
Control	9.2[1]		11.2		7.0			
ZYMV	5.0	46	4.4	61	1.6	77	3.7	60
WMV	8.6	7	8.0	29	4.4	37	7.0	23
WMV + ZYMV	4.2	54	4.2	63	1.8	74	3.4	63
Cultivar mean	6.8		7.0		3.7			
LSD (5%)	1.43[2]						2.48[3]	

[1] For comparing treatments for a given cultivar, LSD (5%) = 3.44.
[2] LSD (5%) for comparing cultivar means.
[3] LSD (5%) for comparing treatment means.

ZYMV and mixed viruses each significantly reduced numbers of marketable squash (60 and 63% respectively) (Table 4). WMV did not significantly reduce numbers of fruit/plot. Miyako produced significantly fewer fruit than Delica and Arjehei.

Table 5: Mean number of non-marketable squash (fruit/plot) and percentage increases relative to control from three cultivars inoculated with ZYMV and WMV.

	Cultivars						Treatment mean	
Treatment	Delica	%	Arjehei	%	Miyako	%		%
Control	6.8[1]		6.4		9.2		7.5	
ZYMV	10.0	47	10.2	59	12.8	39.1	11.0	47
WMV	5.8	15	6.4	0	11.6	26.1	7.9	7
WMV + ZYMV	9.6	41	10.0	56	12.8	39.1	10.8	45
Cultivar mean	8.0		8.2		11.6			
	1.68[2]						2.58[3]	

[1] For comparing treatments for a given cultivar, LSD (5%) = 3.77.
[2] LSD (5%) for comparing cultivar means.
[3] LSD (5%) for comparing treatment means.

ZYMV and mixed virus infections each significantly increased the number of non-marketable fruit (47 and 45% respectively). WMV did not significantly affect the number of non marketable fruit (Table 5).

Table 6: Mean size of fruit (kg) with and without virus symptoms for three cultivars of squash and percentage reduction in size relative to control.

	Cultivars					
	Delica	%	Arjehei	%	Miyako	%
Control (marketable)	2.1		1.7		1.6	
Non marketable (no symptom)	1.8	13	1.6	5	1.3	21
Non marketable (symptom)	0.9	55	0.8	50	1.0	40

Fruit with virus symptoms are significantly smaller than marketable fruit. Viruses had a greater effect on Delica and Arjehei (55 and 50% reduction respectively compared with Miyako (40% reduced)) (Table 6).

Table 7: Mean number of fruit/plot with virus symptoms for three cultivars inoculated with ZYMV and WMV and percentage of total fruit with virus symptoms (square root transformed).

	Cultivars						Treatment mean	
Treatment	Delica	%	Arjehei	%	Miyako	%		%
ZYMV	5.2	33	7.4	53	7.0	49	6.5	45
WMV	1.2	10	1.4	12	4.2	28	2.2	16
WMV + ZYMV	6.2	44	7.4	55	7.8	50	7.1	50
Cultivar mean	4.2	29	5.4	40	6.3	42		

The number of fruit with virus blister symptoms was significantly higher in ZYMV and mixed virus infections than WMV, although the number of fruit with WMV was still higher than in the control (1.2% of the fruit from control plots showed virus symptoms). Miyako is significantly more susceptible to WMV than Delica and Arjehei.

5 DISCUSSION

Currently, the significant viruses of buttercup squash are still confined to the Hawke's Bay, albeit at lower levels than in previous years. WMV continues to be the predominant disease, displacing ZYMV completely in squash again this season. ZYMV has not disappeared completely, as indicated by the weed and volunteer survey results, leaving the possibility of an outbreak in the future. Continued monitoring of the national crop should continue for another season to keep growers informed of any changes in disease status.

Of continued concern is the potential for a disease carry-over of both viruses from seed in infected fruit, particularly if seed continues to survive over a number of seasons, as it appears to do. Once again, further work is needed on this process of transmission.

Aphid vectors prevailed early in the growing season (September-October) and again during February-March. At this stage, information on aphid vectors is still not complete. There is also evidence that other aphid species associated with infected weeds may be involved in disease spread.

We also found evidence of a clear relationship between virus in a previous squash crop, associated weeds, and subsequent infection in a current squash crop.

The effects of mid-season inoculation on Delica, Arjehei and Miyako confirm last season's observations. ZYMV and mixed infections caused greatest losses to yield, quality and fruit number. WMV was less significant since only early season infections cause real losses (Fletcher 1996). It was observed that Miyako could be more sensitive to losses than the other two cultivars.

6 RECOMMENDATIONS

The current research programme should continue at least until the year 2000 with an emphasis on the following areas.

- Continuing ecological studies to determine alternative disease hosts and vectors so that steps can be taken to minimise any virus survival.
- Continuing to monitor the national crop for any spread of disease.
- Monitoring and understanding the processes of seed transmission.
- Continuing to define alternative disease management methods.
- Screening virus-resistant squash crosses.
- Continuing to define aphid flight patterns.
- Monitoring specific crops to develop a simple and appropriate aphid scouting programme.
- Working with interested growers to prepare an application for an AGMART Progressive Farming Grant to fund an aphid monitoring programme in Hawke's Bay.

7 ACKNOWLEDGEMENTS

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