Buttercup squash

- virus disease research report 1995/96

A report prepared for

the New Zealand Buttercup Squash Council

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CONTENTS

1 EXECUTIVE SUMMARY

A national virus disease survey of squash crops was completed during the 1995/96 growing season. Watermelon mosaic virus (WMV) was active only in Hawke's Bay and present around Gisborne. No zucchini yellow mosaic virus (ZYMV) was detected in squash crops. Mid-season infections of WMV developed in Hawke's Bay to an estimated incidence of 30% in some crops, whereas the incidence of early and late season infections was never more than 10%. Weed hosts in old squash sites were found to be infected with both viruses, as were some volunteer seedling squash plants. These observations suggest that the potential disease reservoir is wide.

Aphid vectors of the viruses were found to be flying from October through to March, although in low total numbers. Seed transmission of WMV and ZYMV was detected in saved seed from infected fruit at levels of 7% and 1% respectively.

A yield trial determined that early and mid-season infection of plants with either virus reduced the number and weight of marketable squash fruit, and the total number of fruit produced by those plants. Late season infection did not affect these yield parameters. Similarly, fruit blister symptoms of virus infection were most common with early and mid-season infections. In addition to ZYMV, WMV was found to also cause blistering. In most treatments, infections with ZYMV alone caused more severe losses of marketable fruit than WMV or a mixed infection.

Work will continue over the next two seasons to monitor the national crop for disease spread; determine alternate disease hosts; understand processes of seed transmission; further define aphid flight patterns; explore the development of fruit symptoms in WMV; screen virus-resistant squash crosses; and further define appropriate disease management methods.

2 INTRODUCTION

The outbreak of virus disease in Hawke's Bay in 1994/95 drew attention to the vulnerability of the New Zealand squash crop to damage from such diseases. Reaction from the industry to this challenge was swift and effective. The publication of information about the diseases, watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV), with appropriate control measures, enabled growers to make informed decisions on the diseases. Grower meetings were held to discuss with exporters and packers ways to contain and handle affected crops. Research projects funded by the New Zealand Buttercup Squash Council helped define the extent of the diseases (Fletcher & Jermyn 1995) and the importance of their aphid vectors (Herman 1995). Following this, funds were allocated from the Public Good Science Fund (PGSF) (96-CRF-04-4937 Diseases of arable and vegetable crops) for research on disease identification, effects, spread and control. In addition, the Squash Council allocated funds, under their Crop Management research priority, for specific research on monitoring aphid vector flight patterns, disease spread and overwintering of viruses in weeds and volunteer squash.

This report summarises research on virus disease management in squash funded both by the Public Good Science Fund (PGSF) and New Zealand Buttercup Squash Council during 1995/96.

3 METHODS

All virus diseases were identified and confirmed using serological assays (ELISA). Where appropriate, specific virus isolations were also made using differential indicator plants.

3.1 National squash virus disease survey

A national survey of squash crops in the main squash-growing regions was undertaken from December 1995 to February 1996. In consultation with the New Zealand Buttercup Squash Council National Delegates, local contacts were made and samples of regional squash crops were surveyed. If virus disease was observed in any region early in the season a second visit was arranged.

Eight regions were covered in the survey: Northland (Dargaville, Wellsford), Auckland (Pukekohe, Mangatangi), Bay of Plenty (Te Kaha), East Coast (Gisborne), Hawke's Bay (Hastings), Manawatu (Palmerston North, Wanganui), Marlborough (Blenheim) and Canterbury (Ashburton, Rakaia).

3.2 Hawke's Bay squash virus monitoring

In 1995, the region most affected by mosaic virus diseases was Hawke's Bay. In addition to the national crop survey and as a supplement to the disease epidemiological programme, various Hawke's Bay squash crops were monitored. Three groups, early, mid, and late harvest were chosen to represent as much of the main growing area in Hawke's Bay as practicable.

A small organic block was planted adjacent to the aphid traps at the Crop & Food Research Lawn Rd station and monitored for disease development and spread.

3.3 Disease epidemiology

To understand further how squash viruses survive and spread in New Zealand crops, a number of approaches have been used.

3.3.1 Weed survey

Overwintering weeds in and around squash crops that showed disease symptoms the previous season were collected and assayed for virus infection.

3.3.2 *Squash volunteers*

Volunteer squash seedlings, collected from squash crops that showed disease symptoms the previous season, were assayed for virus infection.

3.3.3 *Seed transmission*

The seed from virus infected fruits from a Hawke's Bay crop was sown, and the resulting seedlings were assayed for WMV and ZYMV. Further seeds from infected fruit were dissected and assayed for virus infection using ELISA.

3.3.4 *Aphid vectors*

Aphid flight patterns were monitored at Lawn Rd from November 1995 to March 1996. Yellow sticky traps were collected weekly from two height positions (1 m and 2.5 m above ground) adjacent to the small organic crop trial.

3.4 Field trial on the effects of squash viruses on fruit yields

To further understand the potential effects of WMV and ZYMV on squash crop yields a replicated yield trial was established at Lawn Road. Experimental treatments included: single WMV or ZYMV infection, mixed virus infections, and early, mid and late season inoculations. The experimental plots of Delica were sown on 25 November 1995 at 40 cm spacings, 18 plants/plot with 2 m row widths and were managed according to recommended guidelines (King & Wishart 1993). The plots were hand weeded, and plants hand-trained. The crop was sprayed twice for powdery mildew and was harvested, weighed and assessed for quality on 15 February 1996.

4 RESULTS

4.1 National squash virus disease survey

In total, 120 crops of squash, zucchini, melon and other cucurbits were surveyed. No sign of either WMV or ZYMV was recorded in most regions. In Hawke's Bay and Gisborne, WMV infection was recorded early in the season but in Gisborne the disease did not develop further. Only in Hawke's Bay did WMV disease develop through the season and a full monitoring programme was initiated. No ZYMV was detected in any surveyed crop.

4.2 Hawke's Bay squash virus monitoring

Results of squash virus monitoring in Hawke's Bay are summarised in Table 1. Early sown crops developed only trace levels of WMV and sometimes CMV (cucumber mosaic virus). Only one crop developed an estimated 10% incidence of disease. Mid-sown crops appeared to be the most affected by WMV, with trace levels early on, developing to 30% incidence, and in one crop of crown pumpkin, to 80% incidence. Late sown crops, were similar to early sown ones, developing only trace or no more than 4% incidence of WMV. It was noted that two organic crops had only a trace infection of WMV. The small organic crop monitored through the growing season began to develop virus symptoms in late February. Four plants were infected with both WMV and ZYMV. The infection source was most likely to have been the yield trial 300 m to the south.

4.3 Disease epidemiology

4.3.1 Weed surveys

A number of weed species were found to be infected with the viruses WMV, CMV and sometimes ZYMV (Table 2). Some weeds were widespread early in the spring (wild carrot, speedwell, stagger weed, cleavers and red root) both around crop margins and as seedlings within an old crop. Neither clover nor ranunculus were found to be hosts of squash viruses in this survey.

4.3.2 Squash volunteers

Table 3 summarises the viruses detected in the volunteer squash plants surveyed. WMV and ZYMV were detected in 6% and 8% of plants, respectively. CMV was found in 20% of plants.

4.3.3 *Seed transmission*

The assay of seed from infected fruit (cv. Diamo) from a 1994/95 crop showed that ZYMV and WMV were transmitted to young seedling squash. Results showed 7% of seed carried ZYMV and 1% carried WMV, with a mixed infection detected in 0.5 %. Seedlings were usually symptomless. Dissection and assay of seed detected ZYMV and WMV in seed coats, integuments and cotyledons. Infection of the seed embryos was not detected.

4.3.4 *Aphid vectors*

Total sticky trap catches of aphids at Lawn Road are summarised in Figures 1 and 2. Highest numbers of aphids were recorded in late March. Virus vectors were noted from late October through to March. These included *Myzus persicae, Aphis craccivora, Aphis gosypii, Macrosiphum euphorbiae, Cavariella aegopodii,* and *Rhopalosiphum padi.*

4.4 Field trial on the effects of squash viruses on fruit yields

The sequence of inoculations and the stages of plant development throughout the experiment (Section 3.4) are summarised in Figure 3.

Early and mid season inoculations with ZYMV and WMV had a significant effect on the yield of buttercup squash in this experiment. The yield effects measured in this trial are given in Tables 4 to 9. In particular, yield of marketable fruit was reduced by virus infection. Table 4A (marketable weight/plot) shows that there was usually a significant weight reduction. Losses, relative to control yields, of between 54% and 85% were recorded in blemish-free fruit of the marketable weight range, after early, and mid-season infection with the viruses individually, and with both viruses. Mid- and late season inoculation with WMV had no significant effect on yield. Similarly, virus infection reduced the number of marketable fruits/plot (Table 5A) by between 52% and 84%. The total weight of fruit/plot (Table 6A) also followed this pattern but, while significant, the effect was not so severe, with a range of 26-47% reduction in weight due to infection. Total number of fruit (Table 7A) was only slightly reduced with early inoculation, with no effect from mid- and late inoculation. In all cases, early infection with ZYMV reduced yield the most, followed by WMV, then infection with both viruses.

The overall effect of virus inoculation (Tables 4B to 7B) further confirmed the impact of early and mid-season infection. The symptom of fruit blister, an important factor in selecting marketable fruit, was prevalent in early and mid-season inoculated plots (Table 8A) with a range of incidence of 34-74%. The occasional presence of infection with both viruses showed no significant fruit symptoms.

Overall viruses (Table 8B) confirmed the early and mid-season effect. Of particular importance was the clear observation that fruit blisters could also be attributed to WMV

in buttercup squash. Previous field observations had only attributed blisters in squash to ZYMV.

Symptoms of leaf mosaic were widespread in early and mid-season inoculated plants (35-89% of plants showed symptoms).

A further observation was that plant leaf symptoms were less severe when plants were infected with both WMV and ZYMV (Table 9A). In addition, leaf symptoms appeared to be less prevalent over time (Table 9B), perhaps indicating the reduced susceptibility of more mature plants to virus infection.

Table 1: Viruses found in Hawke's Bay squash crops — November 1995 to February 1996.

Location	Date of surveys				
	1/11/95	18/12/95	5/1/96	30/1/96	21/2/96
Early season crops					
Lawn Road, A Havelock North	0	CMV+	0	H	
Te Aute Road, A Havelock North	0	WMV	WMV	H	
Ngataruwa, A	0	0	WMV	H	
Opapa	0	0	WMV[T]	H	
Mid season crops					
Mill Road Havelock North	-	0	0	0	H
Lawn Road, B	-	0	0	0	H
Farndon Road Whakatu	-	0	0	0	H
Pakowai Road Pakowai	-	CMV[T]	-	WMV 5%	WMV 10%
Longlands Road Longlands	-	0	0	WMV[T]	H
Te Aute Road, B Havelock North	-	0	WMV[T]	WMV[T]	H
Te Aute Road, C Havelock North	-	WMV[T]	WMV 5%	WMV 20%	H
Te Aute Road, D	-	0	WMV 10%	WMV 15%	H
Te Aute Road, E	-	WMV[T]	WMV 5%	WMV 25%	H
Te Tua Organic	-	0	0	WMV 1%	H
Te Tua	-	0	0	0	H
Ngatarawa, B	-	0	WMV[T]	WMV 0.5%	H

Ngatarawa, C	-	0	WMV[T]	WMV 5%	H
Ormond Road Twyford	-	0	0	0	H
Nicholls Road Twyford	-	-	WMV 15%	WMV 20%	M
Swamp Road Pitoitoi	-	-	WMV[T]	WMV[T]	H
Irongate Road (pumpkin) Hastings	-	WMV 30%	WMV 40%	WMV 80%	H
Longlands Road (butternut)	-	-	WMV 1%	WMV 10%	WMV 30%
Pukekura Road, A Te Aute	-	-	0	0	0
Late season crops					
Pukekura Road, B Te Aute	-	-	0	0	0
Pukekura Road C	-	-	0	0	WMV 2%
D	-	-	0	0	0
F	-	-	0	0	0
Tikokino	-	-	0	0	0
Te Aute Road F Havelock North	-	-	0	0	0
Ngatarawa D	-	-	0	0	0
Ngatarawa organic	-	0	0	WMV[T]	WMV[T]
Ngatarawa (zucchini)	-	-	0	0	WMV 2%
Ngatarawa organic (zucchini)	-	-	0	0	WMV 4%

H=Harvested.

*=CMV was sometimes associated with a 'similar to hormone damage' leaf symptom.

T='trace' or isolated plants with infection <1%.

Table 2: Viruses in weeds in and around old squash crops in Central Hawke's Bay 1995/1996

Location	Weed host		Viruses
Irongate Rd, Hastings	Wild carrot	*Daucus carota*	WMV, CMV
	Broadleaved plantain	*Plantago major*	WMV, CMV
	Cornbind	*Polygonum convulvulus*	CMV
Te Aute Rd, Havelock Nth	Speedwell	*Veronica* sp.	CMV
	Black nightshade	*Solanum nigrum*	WMV, AMV
Lawn Rd, Havelock Nth	Hemlock	*Conium maculatum*	WMV
	Cleavers	*Galium apavine*	WMV, ZYMV, CMV
	Fennel	*Foeniculum vulgare*	WMV, CMV
	Horehound	*Marrubium vulgare*	WMV, CMV
	Prickly sow thistle	*Sonchus asper*	CMV
	Redroot	*Amaranthis* sp.	CMV, WMV
Pukekura Rd, Te Aute	Staggerweed	*Stachys arvensis*	ZYMV, CMV, WMV
	Field madder	*Sheradia arvensis*	WMV
	Oxtongue	*Pioris echiodes*	CMV
	Speedwell	*Veronica* sp.	CMV
	Thorn apple	*Datura stromonicum*	CMV
Lawn Rd, Research Station	Ox tongue	*Picris echoides*	CMV, WMV
	Prickly sow thistle	*Sonchus asper*	CMV
	Hawks beard	*Crepis* sp.	WMV

Table 3: Viruses detected in 64 volunteer squash plants in and around old squash crops in Central Hawke's Bay 1995/1996.

Location	Viruses
Te Aute Rd, Havelock North	WMV
Lawn Rd, Havelock North	WMV, CMV, ZYMV
Pukekura Rd, Te Aute	WMV, CMV, ZYMV
Stock Rd, Flaxmere	CMV
Ngatarawa	CMV, ZYMV, WMV
	Of 64 squash plants tested, 13 (20%) were infected with CMV, 5 (8%) with WMV and 4 (6%) with ZYMV.

Table 4A: Mean weight of marketable squash fruit/plot from plots inoculated with different viruses (kg and % reduction relative to control).

	Time of inoculation				
Treatment	Early	%	Mid	%	Late
Control	43.3				
ZYMV	6.6	85	14.5	66	49.5n[1]
WMV	10.9	75	37.4n		38.4n
ZYMV and WMV	18.8	56	19.8	54	36.1n
LSD to a 5% level among treatment 15.2 (LSD P<0.05)					
LSD to a 5% level treatment vs control 12.4					

[1]= not different (P>0.05) from control.

Table 4B: Overall virus effect (on mean weight of fruit for different inoculation times and % of control).

Time of Inoculation	kg	% of reduction relative to control
Control	43.3	
Early	12.1	72
Mid	23.9	45
Late	41.3n	

Table 5A: Mean number of marketable squash fruit/plot from plots inoculated with different viruses and % reduction relative to control

Treatment	Time of inoculation				
	Early	%	Mid	%	Late
Control	21.5				
ZYMV	3.3	84	7.0		23n[1]
WMV	6.0	72	17.6n		18.6n
ZYMV and WMV	10.3	52	10.3		18.0n

LSD P<0.05 = 6.6 among treatments

LSD P<0.05 = 5.4 between control vs treatment

[1]n= not different (P>0.05) from control

Table 5B: Overall virus effect - on mean number of fruits from different inoculation times.

Time of inoculation	kg	% of reduction relative to control
Control	21.6	
Early	6.6	69
Mid	11.7	45
Late	19.9n	

Table 6A: Mean total weight of squash fruit/plot from plots inoculated with different viruses (kg) and % reduction relative to control.

Treatment	Time of inoculation				
	Early	%	Mid	%	Late
Control	51.5				
ZYMV	27	47	38	26	62.5n[1]
WMV	3.8	32	58.8n		53.8n
ZYMV and WMV	38.9	34	37.9	26	49.8n

LSD P<0.05 = 16.8 among treatments
LSD P<0.05 = 13.8 control vs treatment

[1]n= not different (P>0.05) from control

Table 6B: Overall virus effect - on mean total weight (kg).

Time of inoculation	kg	%
Control	51.5	
Early	32.5	37
Mid	44.9	13
Late	55.4n	

Table 7A: Mean total number of squash fruits/plot and % reduction relative to control, from plants inoculated at different times with viruses.

	Time of inoculation				
Treatment	Early	%	Mid	%	Late
Control	29.6				
ZYMV	25.7	15	27.3	7	32.3n[1]
WMV	26	12	35n		30.7n
ZYMV and WMV	27.7	6	26	12	28.7n

LSD P<0.05 = 8.7 among treatments

LSD P<0.05 = 7.1 control vs treatment

[1]n= not significantly different

Table 7B: Overall virus effect - on mean number of fruits/plot for different inoculation times.

Time of inoculation	kg	% of reduction relative to control
Control	29.6	
Early	26.4	11
Mid	29.4n	
Late	30.6n	

Table 8A: Mean % of squash fruits with blister symptoms (mean number of fruits, angular transformed).

	Time of inoculation					
		Early	Mid		Late	
	%			%		%
Control	9	(17.6)				
ZYMV	74	(59.4n[1])	(51.4)	61	(22.2n)	14
WMV	65	(53.6n)	(35.7)	34	(26.4n)	20
ZYMV and WMV	56	(48.6n)	(42.6n)	46	(25.2n)	18

LSD P<0.05 = 13.4 among treatments

LSD P<0.05 = 10.9 treatment vs control

[1]n = not significantly different vs control.

Table 8B: Effect of time of inoculation on % of fruits with symptoms (mean number angular transformed).

Time of inoculation	%	
Control	9	(17.6)
Early	67	(54.8)
Mid	50	(43.2)
Late	17	(24.3)

Table 9A: Mean % of squash plants with virus symptoms in plots inoculated with different viruses (mean number of plants, angular transformed).

	Time of inoculation						
	Early		Mid		Late		
Treatment	%		%		%		%
Control	7	(15.4)	7				
ZYMV	89	(70.9)	89	(51.6)	61	(31.1)	26n[1]
WMV	50	(45)	50	(32.5)	29	(28.3)	22n
Both	50	(44.9)	50	(36.2)	35	(40.6)	42n

LSD P<0.05 = 18.9 among treatments

LSD P<0.05 = 15.5 treatments vs control

[1]n = not significant vs control

Table 9B: Effect of time of inoculation on % of squash plants with virus symptoms (mean number angular transformed.

Time of inoculation	%	
Control	7	15.4
Early	65	53.6
Mid	41	40.1
Late	30	33.1

Fig. 1

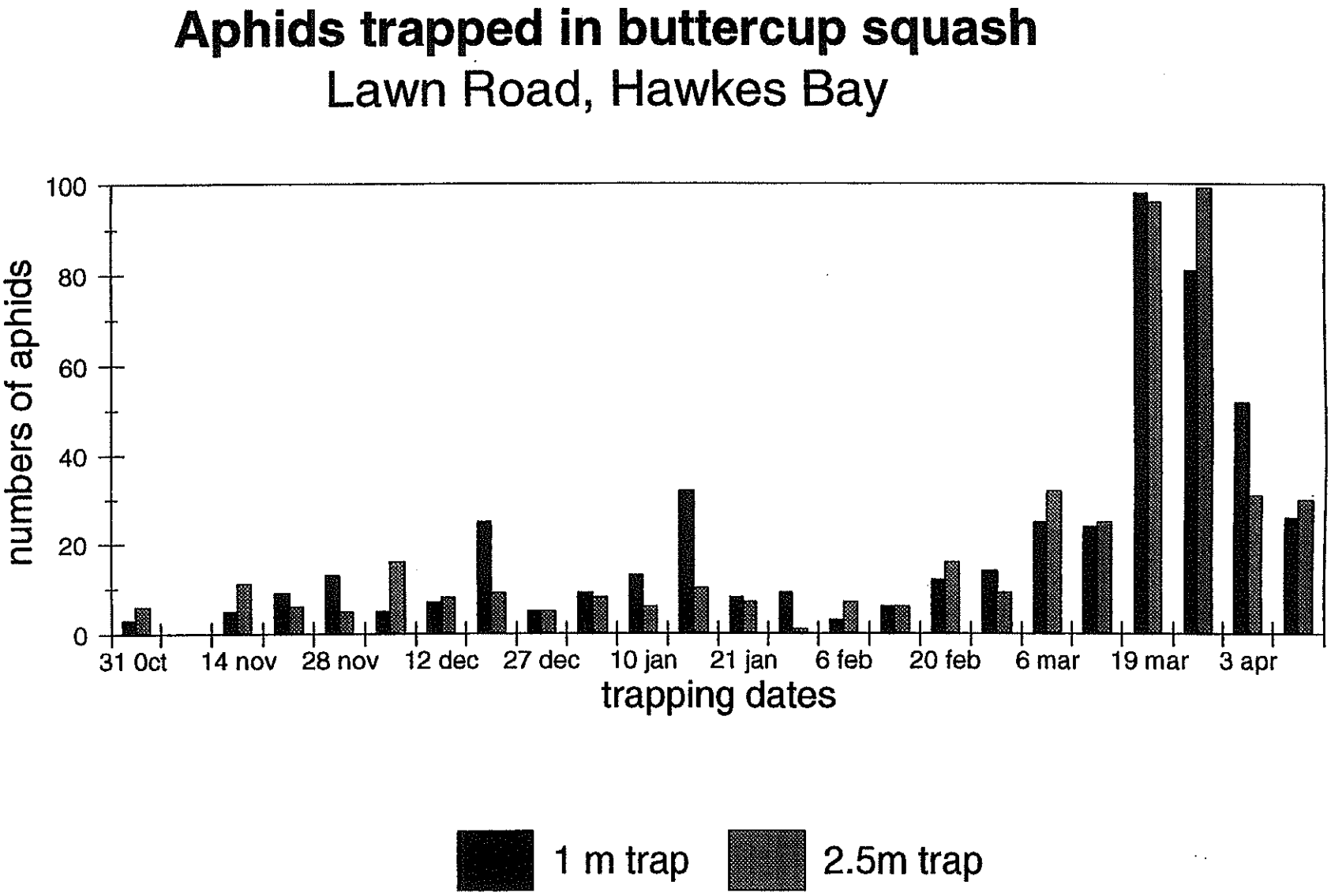
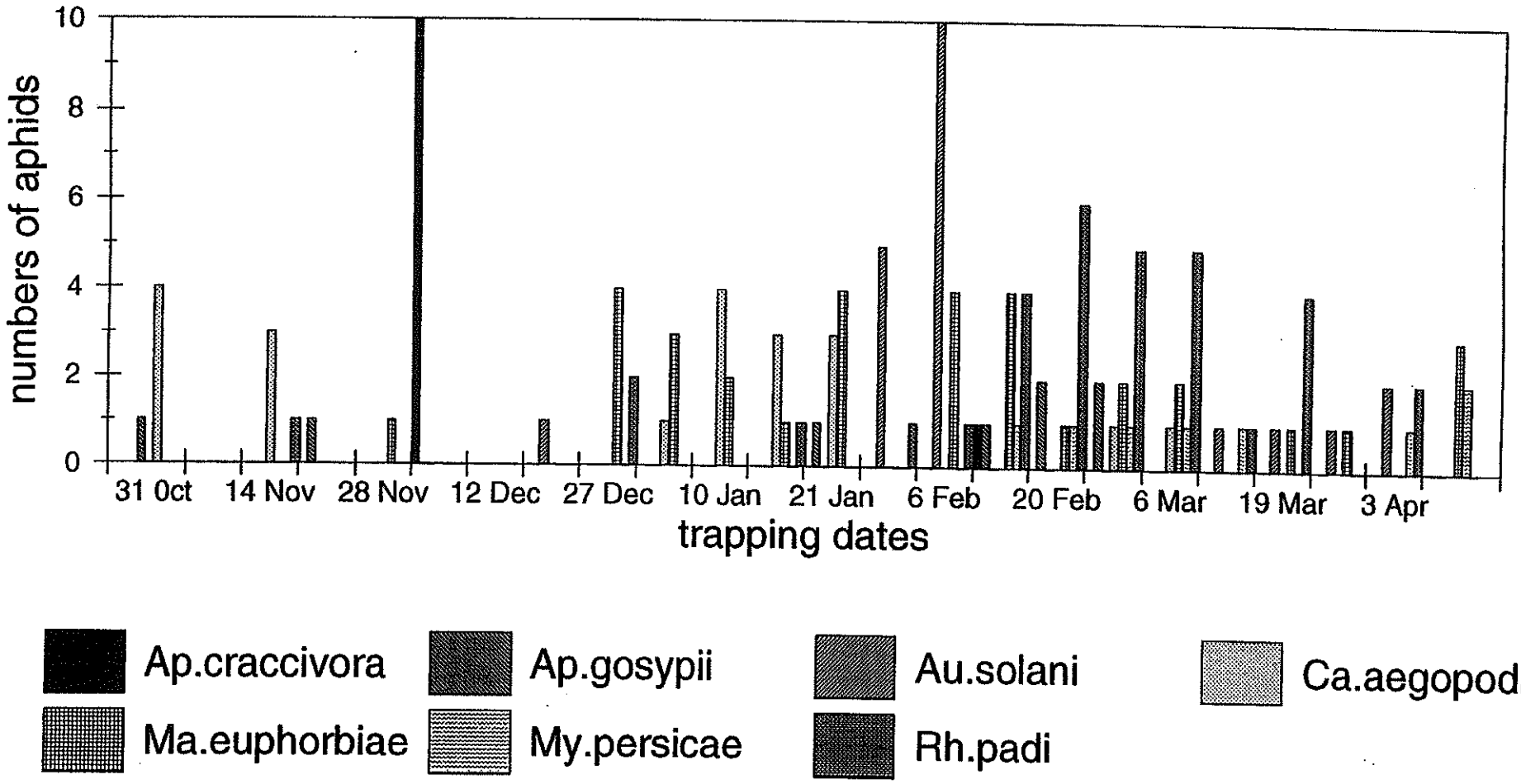

Aphids trapped in buttercup squash
Lawn Road, Hawkes Bay
numbers of aphids
100
80
60
40
20
0
31 Oct
14 nov
28 nov
12 dec
27 dec
10 jan
21 jan
6 feb
20 feb
6 mar
19 mar
3 apr
trapping dates
1 m trap
2.5m trap

Fig. 2

ZYMV & WMV aphid vectors 1995-6

Lawn Rd, Hawkes Bay



	Sowing	Germination	Early inoculation	Mid inoculation	Flowering commenced	Late inoculation	Harvest
Nov 1995	25/11	5/12	19/12	4/1	19/1	30/1	22/2
			Dec		Jan		Feb
Days from sowing	0 days	11	22	39	53	68	91
Growth degree days (GDD)	8.00	141.9	357.7	538.25	706.25	906.55	1284.5

Figure 3: Time line for squash virus yield trials, Lawn Road, Hawke's Bay

5 DISCUSSION

The significant viruses of buttercup squash are confined to the Hawke's Bay, and to a far lesser extent, Gisborne. WMV appears to be the predominant disease, completely displacing ZYMV this season. It is unclear, however, whether or not ZYMV has disappeared completely. It is still possible, on the basis of weed and volunteer squash surveys, that ZYMV could survive in these plants and break out in the future. Further work is needed to determine alternate disease hosts so that steps can be taken to minimise any virus survival. Continued monitoring of the national crop is also required in order to keep growers informed of changes in disease status.

Of continued concern is the potential 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 to fully characterise this process of transmission.

Aphid vectors prevailed throughout the growing season but information on aphid vectors is not complete because the early season patterns of occurrence are not well defined. Other aphid species casually feeding on squash may also be involved in virus spread.

The effects of early and mid-season inoculation with WMV and ZYMV are clear from our experiments. Significant reductions in yield, quality and fruit number have been demonstrated. The role of WMV in causing fruit blisters is not well understood and needs further investigation. Protection of early and mid-season sowings from disease is essential in high risk areas such as parts of Hawke's Bay. It is important to explore the use of early season protection from virus vectors, especially the possible use of seed treatments to prevent aphid feeding. Alternatively, diligent monitoring of vector occurrence within crops and basic hygiene (Fletcher 1995) will assist in disease management.

6 RECOMMENDATIONS

Growers should continue to use published guidelines (Fletcher 1995) for managing squash virus diseases.

The current research programme should continue at least until 1998 with an emphasis on the following areas:

- determining alternate disease hosts so that steps can be taken to minimise any virus survival;
- continuing to monitor of the national crop for spread of disease;
- monitoring and understanding the processes of seed transmission;
- continuing to define aphid flight patterns;
- exploring the development of fruit symptoms caused by WMV;
- continuing to define alternate disease management methods;
- screening squash crosses and breeding material for virus resistance.

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