Incidence of buttercup squash virus diseases and experiments in disease forecasting using aphid flights

—a report on the 1998/99 season

A report prepared for

New Zealand Buttercup Squash Council and AGMARDT

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June 1999

New Zealand Institute for Crop & Food Research Limited
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CropInfo Confidential Report No. 622
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1 EXECUTIVE SUMMARY

This document reports on a squash research project funded by AGMARDT and the New Zealand Buttercup Squash Council (NZBSC), and Public Good Science Fund (PGSF) research conducted during the 1998/99 season.

A group of Hawke's Bay squash growers gained funding from AGMARDT and NZBSC to develop a system to forecast the incidence of squash viruses by monitoring aphid flights over three years. The work is an extension of our previous work on disease epidemiology, which continues as part of the project.

Our PGSF-funded work also continued this season. Early, mid and late sown squash crops were surveyed for virus diseases throughout the season to monitor regional disease spread. Experiments on seed transmission of virus and field surveys of weed hosts furthered our studies on the ecology of virus spread.

The season's findings

- Several new weed hosts of the two squash viruses were recorded: wild turnip (WMV2), wavy-leaved fleabane, mallow and hemlock (ZYMV and WMV2). Aphid species that are potential vectors of the viruses were sometimes recorded on the weeds.

- The incidence of virus was highest early in the season compared to the previous two seasons. Virus incidence then declined over the rest of the season.

- The spring aphid flights (in wind traps) were dominated by an early and exceptionally high peak, compared to the previous two seasons, which included large numbers of three possible vectors; *Rhopalosiphum padi*, *Lipaphis erysimi*, and *Capitophorus eleagni*. The autumn aphid flights (wind traps and suction trap) had a prolonged peak compared to the previous two seasons.

- Aphids were found colonising the crops following the flight peaks in the wind traps. The earliest sown crop of the four sampled had a relatively high aphid population (predominantly *Macrosiphum euphorbiae*) early on which then declined, but increased again late in the growth of the crop. One crop (under organic management) had a low aphid population. In the remaining two crops, aphid populations (*Aphis gossypii*) developed late in the growth of the crops and reached very high levels, particularly in the later planted of the two.

- Virus incidence in the above four crops only exceeded 5% in one crop and that was very late in the growth of the crop. There was no detectable yield loss in the four crops and no signs of fruit damage.

- Aphid catches in the suction trap mainly followed the trend in wind trap catches although more aphids were caught. Potential vector species made up the majority of the catch.

- Seed testing experiments showed that squash viruses, when present, are concentrated in the seed coat and surrounding tissue and occasionally in the embryo.

In conclusion, incident weather plays an important role in the development of aphid populations and subsequent spread of virus diseases. Weed hosts may have a considerable role as sources of inoculum for infection of squash crops. Early results with the suction trap suggest that it may be useful in developing a forecast of virus risk to assist sowing and spraying decisions.

In the coming season we will continue to develop a system for forecasting the incidence of squash viruses, to monitor regional disease spread, and to study the processes of seed transmission and the ecology of virus spread.

2 INTRODUCTION

This document reports on research conducted during the 1998/99 season. There were two aspects to the research: an AGMARDT/New Zealand Buttercup Squash Council (NZBSC) funded project and Public Good Science Fund (PGSF) research.

A group of Hawke's Bay squash growers gained funding from AGMARDT and NZBSC to develop a system to forecast the incidence of squash viruses by monitoring aphid flights over three years. This work is an extension of our previous work on disease epidemiology, which continues as part of the project.

Our PGSF funded work continued this season. Early, mid and late sown squash crops were surveyed for virus diseases throughout the season to monitor regional disease spread. Experiments on transmission of virus and field surveys of weed hosts furthered our studies on the ecology of virus spread.

3 METHODS

3.1 Weed survey

Samples were taken of overwintering weeds in and around the previous seasons' diseased squash crops in Hawke's Bay. Individual plants were collected of species in low abundance, and groups of up to five plants of abundant species were collected. These samples were assayed in the laboratory for possible virus infections of ZYMV (zucchini yellow mosaic virus) and WMV2 (watermelon mosaic virus 2) using serological assays (ELISA) and indicator plants. Aphids feeding on these weeds were also collected and identified as potential virus vectors.

3.2 Squash virus monitoring

Forty-five early, mid and late sown squash crops around Hawke's Bay were surveyed, to estimate the development of virus infection over the growing season. Where possible, crops were visited once a month, three times during the growing period. If disease was observed, a transect across the paddock was walked and 100 plants counted at every second step. The percentage of plants with a mosaic symptom was recorded and the final figure for the crop before harvest was graphed. Up to five specimens of mosaic or fruit symptoms, typical of those being expressed, were taken and assayed to identify the viruses at each visit.

3.3 Aphid vectors and virus disease development

Aphid flight patterns continued to be monitored at Crop & Food Research, Lawn Rd, Hastings, using a wind trap (at a height of 3 m) which was emptied weekly and the aphids counted and identified.

A wind trap (at 2 m) was also placed on the fence-line of squash crops at four grower sites around Hastings: Lawn Rd (Agnew), Ngatarawa (Lawson), Pakipaki (Ostrich), and Pukekura (Brownrigg). Ngatarawa, Pakipaki and Lawn Rd run in a line west to east while Pukekura is 17 km to the south.

The wind traps were emptied weekly (as above) and weekly samples taken to assess aphid populations and virus incidence in the squash crops. Aphids numbers and species were derived from a random sampling of 30 plants counting aphids on up to

10 leaves/plant. Virus incidence was estimated both visually by examining 100 plants weekly, and by fortnightly random sampling and ELISA testing of 50 leaves.

A suction trap (at 7.5 m) was erected at Ngatarawa in late January and run until late April to monitor flight patterns at high levels in order to give early warning of aphid activity. The trap was serviced weekly by the farmer and the contents were sent to the laboratory where aphids were sorted, counted and identified.

3.4 Seed transmission

Experiments were carried out to develop a simple, rapid seed test for laboratory use using a paper binding assay. This test was used to try and determine where in the seed virus was concentrated. Seeds were soaked overnight, split and blotted on to nitrocellulose paper. Dot immuno-binding assays using specific WMV2 or ZYMV antibody were completed and results visually assessed.

4 RESULTS

4.1 Weed surveys

A number of weed species continue to be found carrying WMV2, and ZYMV1 (Table1). Some host species were quite widespread early in the spring (wild carrot, speedwell, stagger weed, cleavers, twin cress, clovers, red root, and mallow) both around crop margins and as seedlings in and around fields that squash had been planted in last season. New virus hosts detected this season, in addition to those of previous surveys, included: wild turnip (WMV2), wavy-leaved fleabane, mallow and hemlock (ZYMV and WMV2). Aphid species associated with some of these weeds (Table 1) included the potential vectors of ZYMV and WMV2: *Myzus persicae* (green peach aphid), *Lipaphis erysimii* (turnip aphid) and *Acythosiphon pisum* (pea aphid) found on mallow.

Table 1: WMV2 and ZYMV recorded in weeds, with associated aphids, in and around squash crops, Hawke's Bay, 1996-9.

Weed Common name	Species	Virus hosted	Aphids hosted
Black nightshade	*Solanum nigrum*	WMV2	
Broad-leaved dock	*Rumex obtusifolius*	WMV2	
Broad-leaved plantain	*Plantago major*	WMV2	
Chickweed	*Stellaria media*	WMV2, ZYMV	
Cleavers	*Galium aparine*	WMV2, ZYMV	*M. euphorbiae, A. pisum, A. kondoi, A. gossypii*
Fathen	*Chenopodium album*	WMV2, ZYMV	*M. euphorbiae, C. aegopodii M. persicae, B. helichrysi A. solani, A. craccivora*
Fennel	*Foeniculum vulgare*	WMV2	
Field madder	*Sheradia arvensis*	WMV2	
Hawks beard	*Crepis* spp.	WMV2	

Hemlock	*Conium maculatum*	WMV2, ZYMV	
Horehound	*Marrubium vulgare*	WMV2	
Lily of the valley vine	*Salpichroa organifolia*	WMV2	
Mallow	*Malva parviflora*	WMV2, ZYMV	*A. pisum, M. persicae, L. erysimi*
Oxtongue	*Picris echioides*	WMV2, ZYMV	*M. euphorbiae*
Rayless chamomile	*Matricaria dioscoidea*	WMV2, ZYMV	*C. aegopodii*
Redroot	*Amaranthus powellii*	WMV2, ZYMV	*A. pisum, M. euphorbiae, M. persicae*
Scrambling speedwell	*Veronica persica*	WMV2	*B. helichrysi, M. persicae*
Sow thistle	*Sonchus oleraceus*	WMV2	*H. lactucae, M. rosae*
Stagger weed	*Stachys arvensis*	WMV2, ZYMV	
Subterranean clover	*Trifolium subterraneum*	WMV2	
Twin cress	*Coronopus didymus*	WMV2	*B. brassicae, A. kondoi, A. craccivora, L. erysimi, A. malvae*
Vetch	*Vicia sativa*	WMV2	*A. gossypii*
Wavy-leaved fleabane	*Conyza bonariensis*	WMV2, ZYMV	
White clover	*Trifolium repens*	WMV2, ZYMV	*M. euphorbiae, A. pisum, C. elaeagni, M. persicae*
Wild carrot	*Daucus carota*	WMV2	
Wild turnip	*Brassica rapa* ssp. *sylvestris*	WMV2	

4.2 Squash virus monitoring

Incidence of virus in 1998-9 was highest early in the season (Fig. 1) in contrast to the previous two seasons where mosaic was highest in mid-season crops.

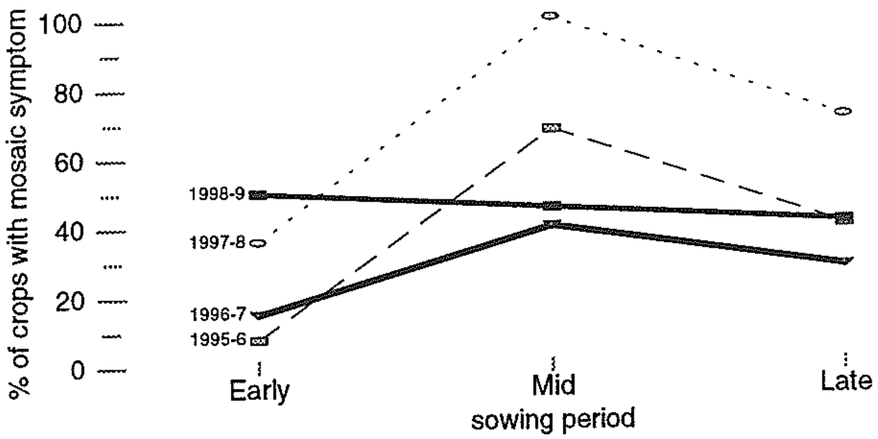


Figure 1: Incidence of virus mosaic in Hawke's Bay squash crops, 1995-1999.

Fifty percent of early sown crops (3 of 6) developed mosaic symptoms (Fig. 2) with the level of virus in each crop ranging between 5 and 20% from the visual estimates. Forty-five percent of mid-sown crops (10 of 22) developed mosaic symptoms with virus levels in each crop ranging from 0.5 to 32%. Forty-one percent of late sown crops (7 of 17) developed mosaic symptoms with virus levels in each crop ranging from 1 to 30%.

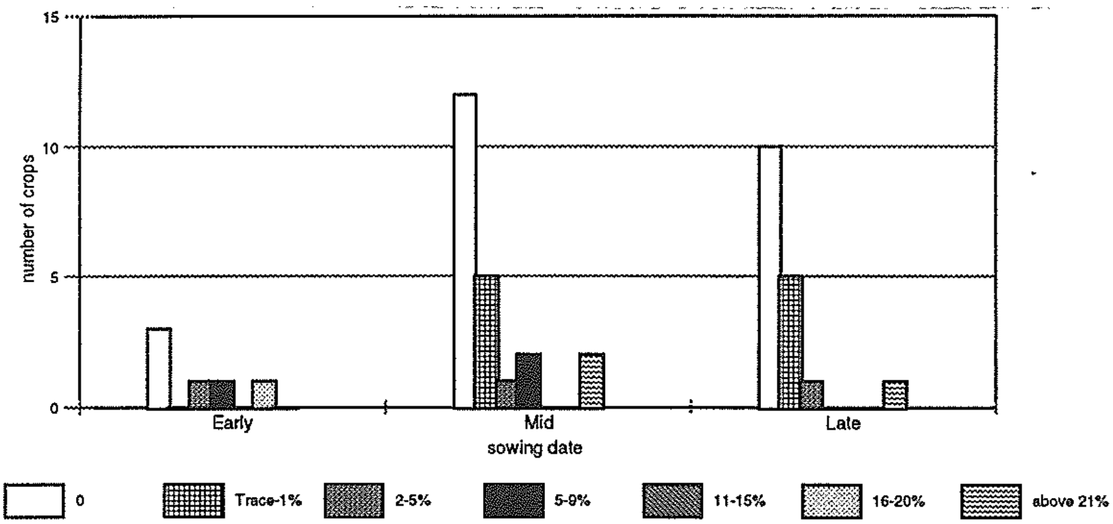


Figure 2: Level of mosaic virus symptoms in early, mid and late Hawke's Bay squash crops 1998/99.

WMV2 was the most widespread mosaic virus, as we have found in previous seasons, with ZYMV recorded only at low levels at various sites. Overall incidence was lower than in most previous seasons with no serious quality losses due to virus reported this season.

4.3 Aphid vectors and virus disease development

4.3.1 Research station wind trap

The pattern of aphid catch (all species) in the research station wind trap (Crop & Food Research, Lawn Rd) was dominated by an early and exceptionally high spring peak and a prolonged autumn peak (Fig. 3) compared to the previous two seasons. The spring aphid flights peaked earlier (mid October) than in the previous two seasons (late October in 1996/97 and late November in 1997/98) and at a much higher peak (436 aphids per week this season compared to 73 aphids per week in 1996/97 and 190 in 1997/98). The spring flights were then cut short by hot windy weather through late spring and early summer.

We also present in Figure 3 the catch of potential vector species (from overseas records) for the past three seasons. The vectors did not make up an equal proportion of the total catch through the seasons. For this season, the spring peak in aphid catch was dominated by three species that are possible virus vectors: *Rhopalosiphum padi* (cereal aphid), *Lipaphis erysimi* (turnip aphid) and *Capitophorus eleagni*.

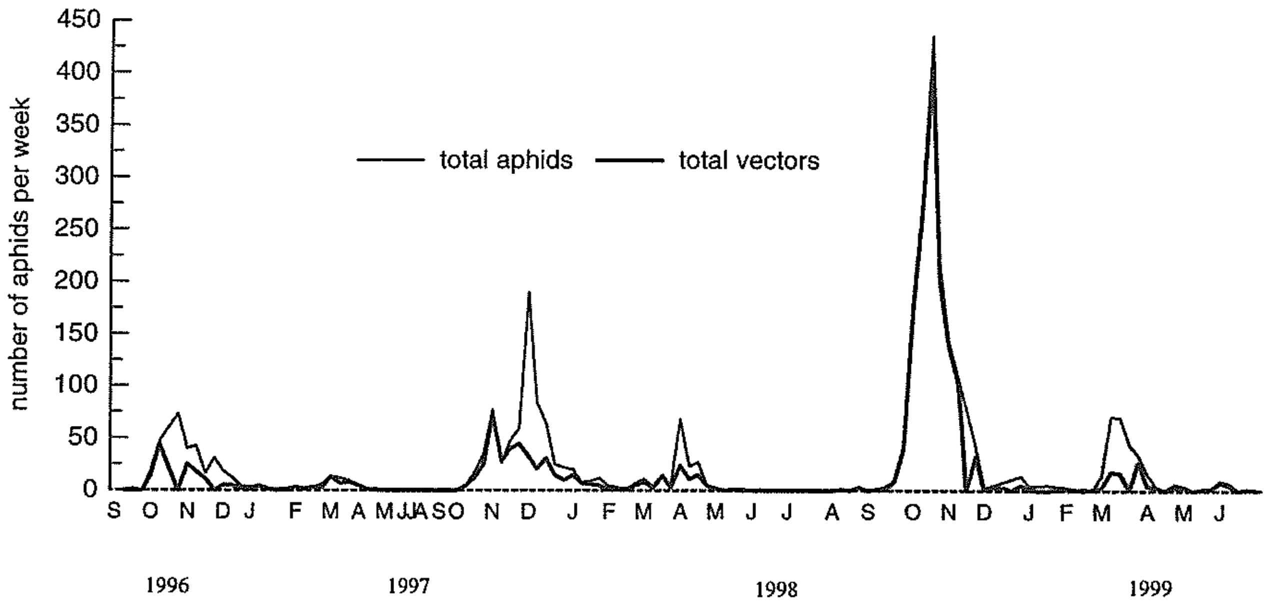


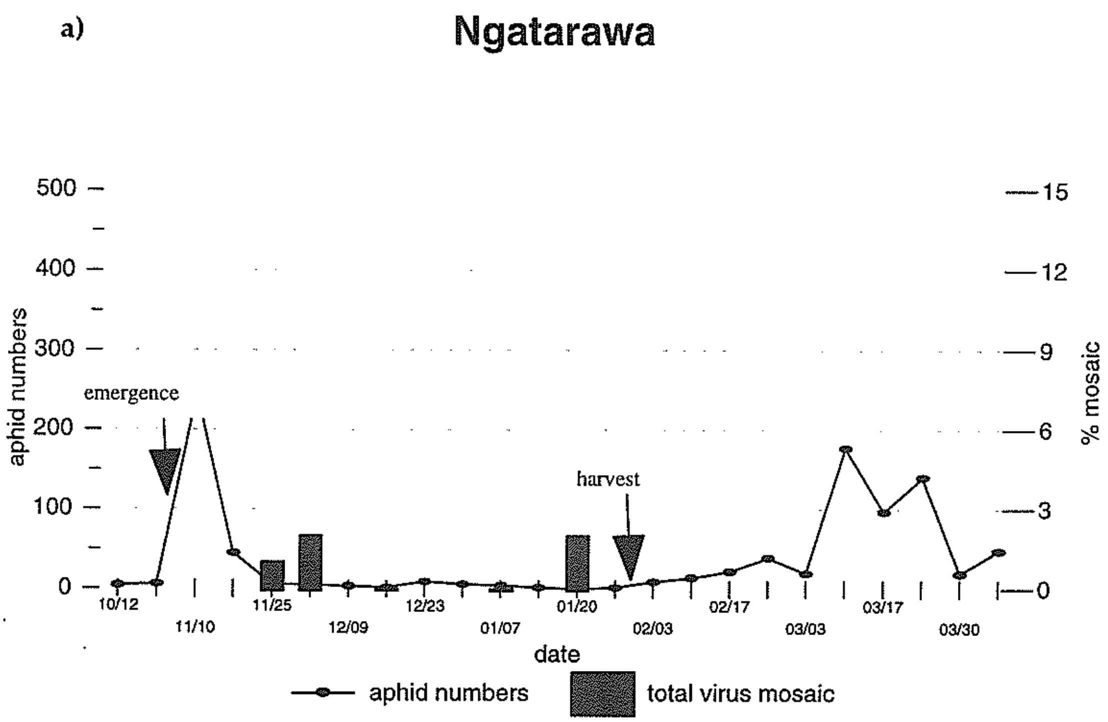
Figure 3: Aphid catch in a 3 m wind trap over three seasons at Crop & Food Research, Lawn Rd Research Station, Hastings.

The prolonged autumn peak in aphid flights was at a similar level to last season's (about 70 aphids per week), but lasted longer because of the milder weather conditions and was not dominated by possible vector species.

4.3.2 *Aphid populations in four squash crops*

Aphid catches in the wind traps in the Ngatarawa, Pakipaki and Lawn Rd crops followed the pattern recorded in the research station wind trap (Fig. 4a, b and c, respectively). Each had a spring peak. This was shortly after crop emergence for Ngatarawa and Lawn Rd and before crop emergence for Pakipaki. The Pukekura crop (Fig. 4d) was not sown until after the spring flights had ceased. The aphid catch in this wind trap was probably related to surrounding, earlier sown squash crops.

a)



b)

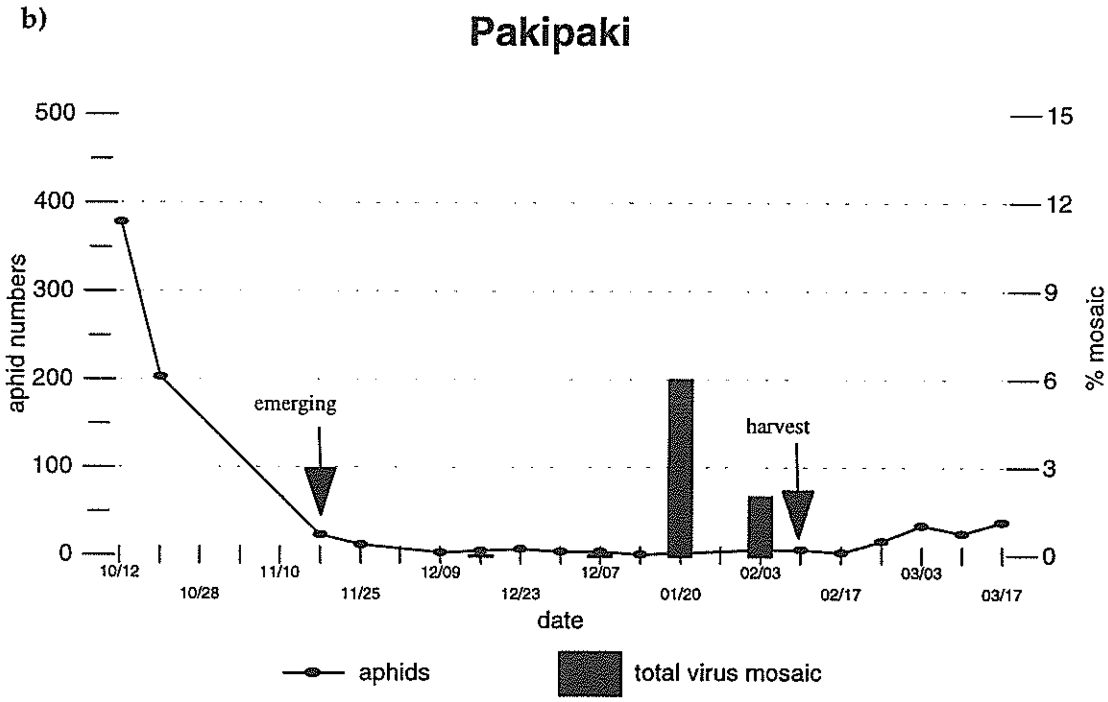
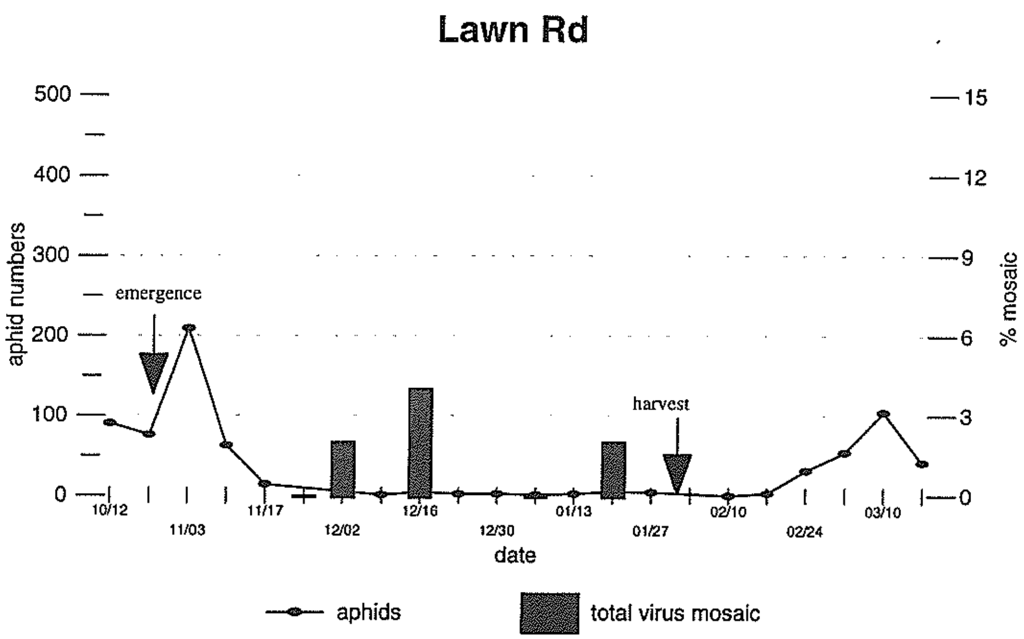


Figure 4: Aphid numbers trapped and total mosaic virus in a) Ngatarawa, b) Pakipaki, c) Lawn Rd, and d) Pukekura crops,1998-9

c)



d)

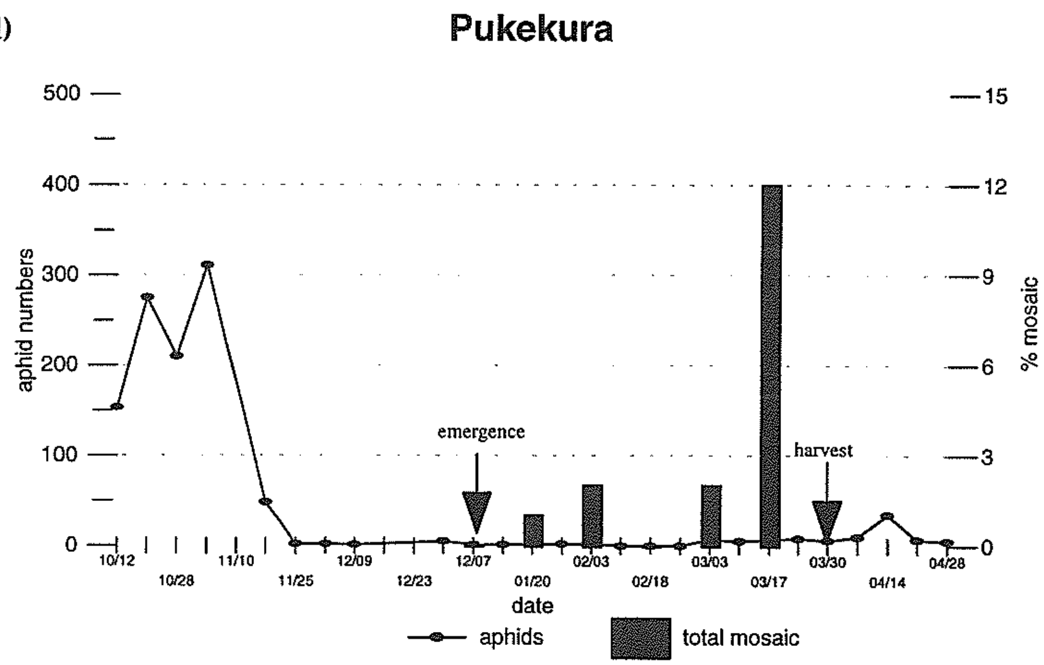


Figure 4: Aphid numbers trapped and total mosaic virus in a) Ngatarawa, b) Pakipaki, c) Lawn Rd, and d) Pukekura crops,1998-9

Autumn flights commenced in late February after the Ngatarawa, Pakipaki and Lawn Rd crops had been harvested. Autumn flights numbers were lower at Pukekura, commencing just before harvest. It was noted that the wind trap in the Pukekura crop was not rotating freely later in the season and this probably affected the aphid catch.

The catch of vector species in the wind traps (data not presented) followed the trend of total aphid catch, but as noted for the research station wind trap, the vectors were not an equal proportion of total aphid catch right through the season.

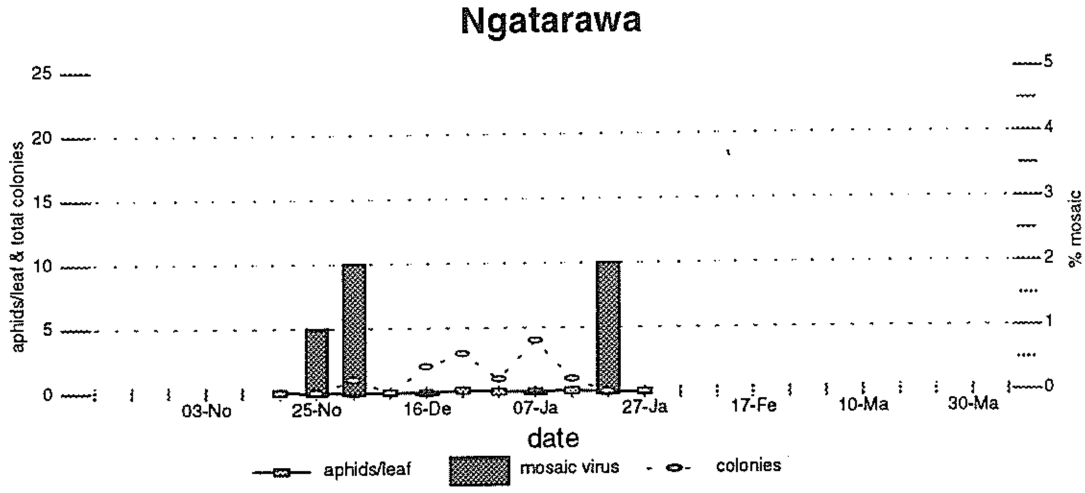
As would be expected, aphids and aphid colonies were found in squash crop plants following flight peak catches in the wind traps.

The aphid population in the Ngatarawa crop (Fig. 5a) remained at low levels throughout the season. The total number of colonies was always less than 5 per 30 plant sample and there was only ever more than one aphid per ten leaves (0.1 aphid per leaf) on one sampling date.

In the Pakipaki crop, aphid populations developed to relatively high numbers (3.4 aphids per leaf) late in the growth of the crop (Fig. 5b). The number of aphid colonies peaked at 26 colonies per 30 plant sample in the same sample.

The Lawn Rd crop was the earliest sown of the four crops and the aphid population was relatively high (3.5 aphids per leaf and 28 colonies per 30 plant sample) just after the crop emerged (Fig. 5c). The aphid population (both aphids per leaf and colony numbers) then declined from this point, but increased again later in the growth of the crop.

a)



b)

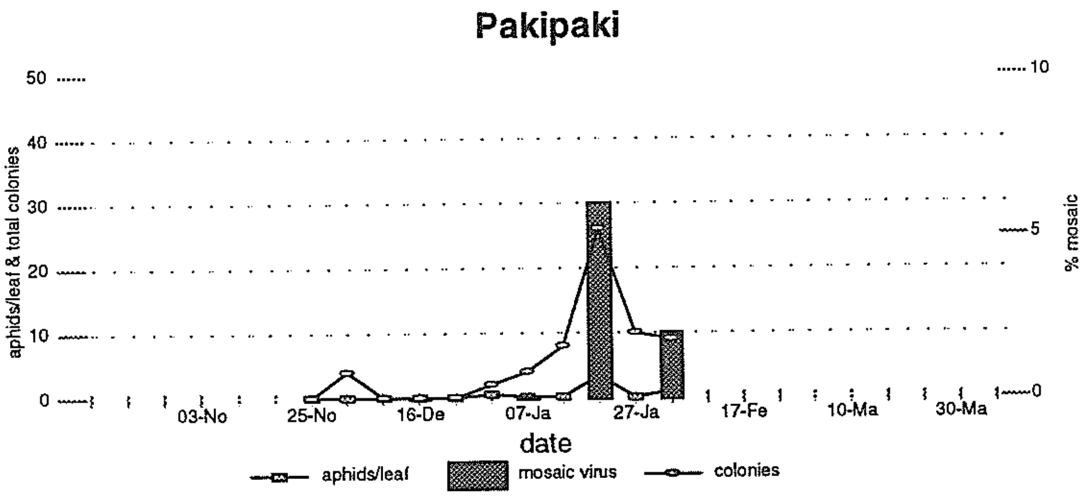
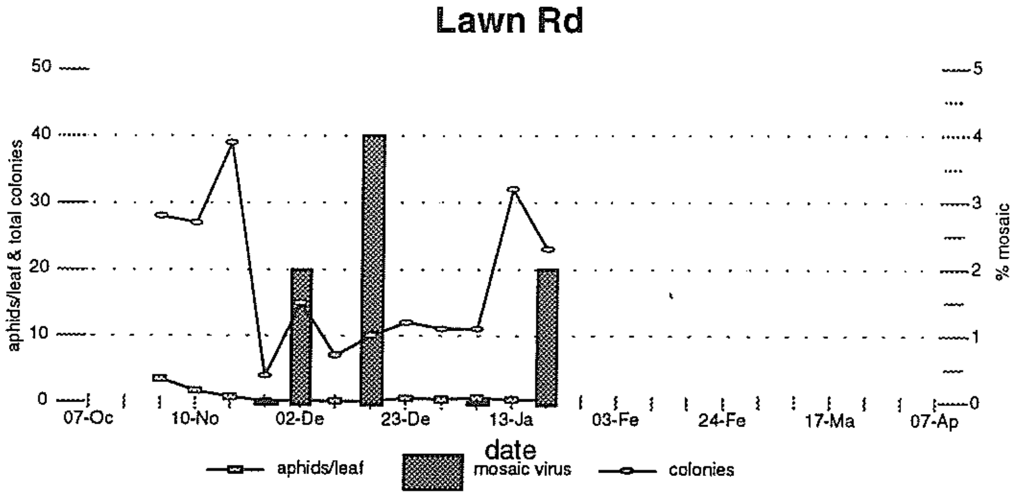


Figure 5: Aphid colonisation of squash crops and associated mosaic incidence in (a) Ngatarawa, (b) Pakipaki, (c) Lawn Rd and (d) Pukekura crops, 1998-9.

c)



d)

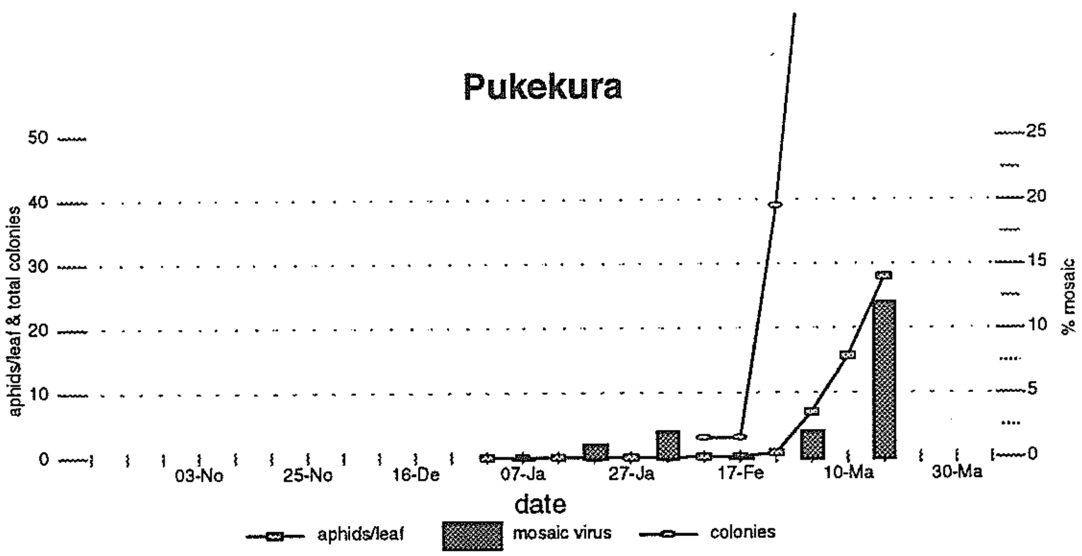


Figure 5: Aphid colonisation of squash crops and associated mosaic incidence in (a) Ngatarawa, (b) Pakipaki, (c) Lawn Rd and (d) Pukekura crops, 1998-9.

The Pukekura crop was the latest sown of the four crops and missed the spring flights of aphids (Fig. 4d). Aphid populations did not develop substantially until late in the season, when there was classical exponential population growth, soaring to 28 aphids per leaf and 1015 colonies per 30 plant sample on the final sample date.

The species colonising the four squash crops was not the same in each crop. *Macrosiphum euphorbiae* (potato aphid) was the dominant aphid in the Lawn Rd crop while *Aphis gossypii* (melon aphid) dominated the aphid populations of the Pakipaki and Pukekura crops. *Myzus persicae* (green peach aphid), the other aphid species that colonises squash, was present in low numbers in most of the crops. Other winged species trapped on plants included recorded vectors: *Aphis craccivora, Lipaphis erysimi, Aulacorthum solani, Cavariella aegopodii, Capitophorus eleagni, Acyrthosiphon kondoi* and *Rhopalosiphum padi*. *Acyrthosiphon pisum*, found colonising squash last season, was not detected in crops this year.

4.3.3 *Ngatarawa suction trap*

Aphid catch in the suction trap largely followed the trend of wind trap catches (Fig. 6), although a larger number of aphids were caught in the suction trap. For both traps the possible vector species made up the majority of the catch as shown for the suction trap (Fig. 7).

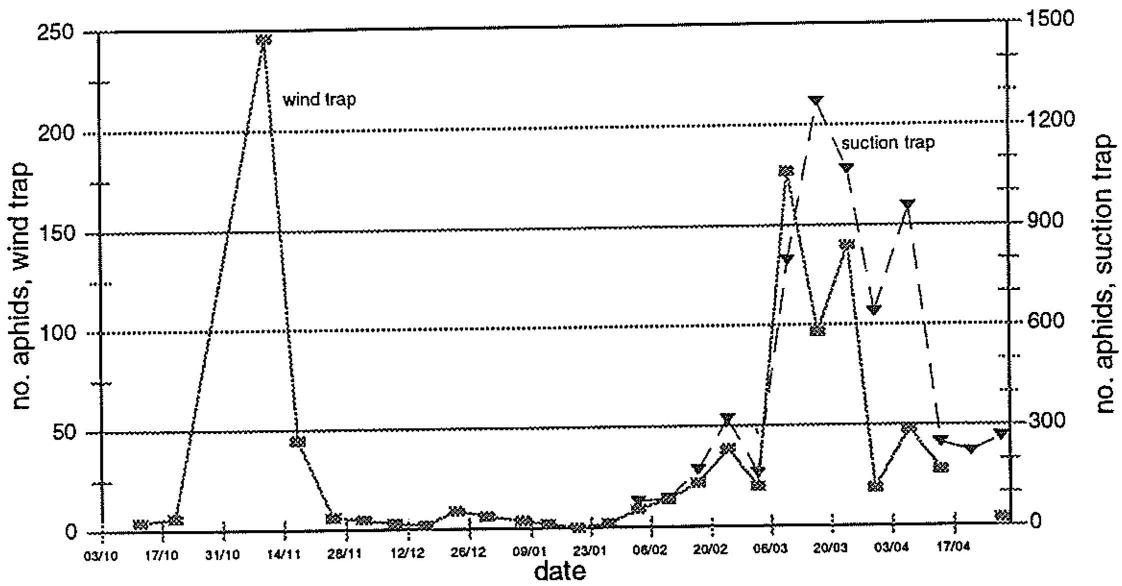


Figure 6: Total number of aphids trapped in a 2 m wind trap and 7.5 m suction trap at Ngatarawa 1998-9.

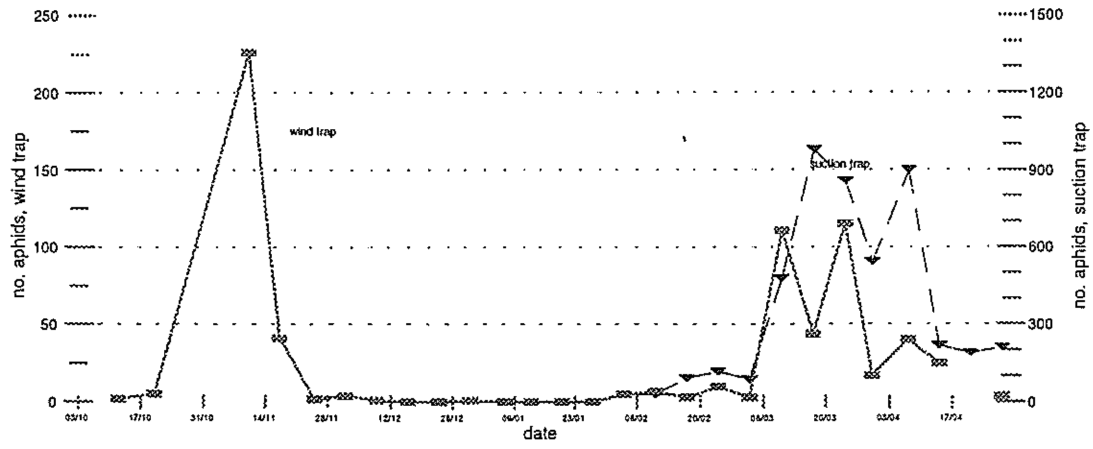


Figure 7: Total number of aphid vectors trapped in a 2 m wind trap and 7.5 m suction trap at Ngatarawa 1998-9.

4.3.4 Virus disease development in four squash crops

In spite of relatively high aphid numbers, mosaic development at the four sites was modest (Fig. 5) and did not lead to any detectable crop loss. At each site maximum virus detected from field sampling was as follows:

- Lawn Rd 2% each for ZYMV and WMV2;
- Pakipaki 4% ZYMV and 2% WMV2;
- Ngatarawa 2% ZYMV;
- Pukekura 2% ZYMV and 10% WMV2.

Visible mosaic was noted at Pakipaki (4%), Lawn Rd (5%), Ngatarawa (trace) and Pukekura (4%). There was no sign of any fruit damage. Peaks in virus incidence occurred 2 weeks after peaks in numbers of aphid colonies. Virus incidence sometimes appeared to drop away, but possibly our sampling did not always detect the comparatively low levels of virus disease seen this season.

4.4 Seed transmission

Our seed testing experiments were successful in detecting virus in individual seed. Virus, if present, appears to be concentrated in the seed coat and surrounding tissue and only occasionally in the embryo. One line of oilseed squash was found to have 4% WMV2 and 1.5% ZYMV seed infection. When sown and grown this crop developed 32% mosaic, the highest level recorded this season. There was no sign of obvious fruit damage although total yields were probably down.

5 DISCUSSION

Our studies this season continue to show that WMV2 is the major cause of mosaic symptoms in Hawke's Bay squash crops. ZYMV is still present but has not damaged crops seriously as in the past. However, in three of four closely monitored crops ZYMV was as prevalent or more prevalent than WMV2. In general mosaic symptoms were only a minor problem this season. This was in spite of spring aphid flights being at the highest levels we have so far recorded. Incidence of mosaic infection in early crops was similar to last season's pattern. This trend ceased when the hot dry weather during November and December suppressed aphid flights earlier, thus reducing the risk of disease spread from weeds and early infected crops.

Aphids, once established, managed to build up in some crops even under the hot dry conditions. In one early and one late crop, mosaic levels reached 30%, though there was no sign of fruit damage. Colonisation appeared to start when aphids reached a figure of around 0.2 aphids/leaf. Further statistical analysis is being undertaken to confirm these observations as a means of further guiding growers in their decision making.

No new aphid species were found colonising crops this season. It was interesting to note the lowest levels of aphid build-up occurred in two crops with contrasting control methods: one (Ngatarawa) relied on natural predators in an organic situation; the other (Pukekura) used Gaucho seed treatment. However, the late planting of the latter crop, which missed the spring flights, may also have been a contributing factor.

The successful establishment of the suction trap at Ngatarawa is expected to give growers a more reliable measure of early aphid flight movements. Suction traps provide a continuous, unbiased random sample (Tatchell 1990) by drawing a continuous volume of air through to actively catch aphids. Wind traps only passively catch aphids; they rely on some wind to turn the trap to face into the wind and aphids being carried along in the wind may be caught. Suction traps catch aphids flying at high levels. These aphids are true migrants and are not influenced by ground factors. Aphids caught in the lower level wind traps respond to ground factors which can, in turn, bias the catch in the wind trap.

Our autumn results indicate that the suction trap catch is comparable to that in the wind traps. If this relationship between the suction trap and wind traps reoccurs in the spring we can be confident of being able to develop a forecast, based on aphid catch in the suction trap, to support sowing and spraying decisions for squash crops.

New weed hosts of virus continue to be found, along with their associated aphids. WMV2 is known to have a wide host range but it is interesting that around Hawke's

Bay we are still finding new hosts for ZYMV. Overseas, most weed hosts for this virus are confined to the family Cucurbitaceae. Here, the virus seems to infect many other weed families in its quest to overwinter.

5.1 Plans for the coming season

In the coming season we will continue research to develop a system to forecast the incidence of squash viruses (AGMARDT/NZBSC funded). This will include setting up a website and grower fax-out to notify local growers of the levels of aphid flights. Our PGSF funded research will continue to monitor regional disease spread through the season and study the processes of seed transmission and the ecology of virus spread.

6 ACKNOWLEDGMENTS

We wish to thank Sarah Legg and Brian Rogers for their skilled technical assistance. We also thank AGMARDT, NZBSC, the Foundation for Research, Science and Technology and the contributing growers for both financial and 'in kind' support of this project.

7 REFERENCES

Fletcher, J.D. ; Rogers, B.T.; Wallace, A.R. 1997: Buttercup squash - virus disease report 1996/7. Cropinfo confidential report No 419. Crop & Food Research, Lincoln. 17 p.

Fletcher, J.D. 1998: Buttercup squash - virus disease report 1998. Cropinfo confidential report No. 297. Crop & Food Research, Lincoln. 22 p.

Tatchell, G.M. 1990: Monitoring and forecasting aphid problems. *In*: Peters, D.C., Webster, J.A., Chlouder, C.S. (eds). Proc. Aphid-Plant interactions: populations to molecules. USDA/ARS, Oklahoma State Univ. 215-231