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Scoping study on the importance of virus diseases in Australian vegetable cucurbit crops

Brenda Coutts
Department of Agriculture
Western Australia

Project Number: VG03057

VG03057

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 1101 0

Published and distributed by:
Horticultural Australia Ltd
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50 Carrington Street
Sydney NSW 2000
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FINAL REPORT

HORTICULTURE AUSTRALIA PROJECT VG03057

SCOPING STUDY ON THE IMPORTANCE OF VIRUS DISEASES IN AUSTRALIAN VEGETABLE CUCURBIT CROPS

Ms B A Coutts, *et al*.
Department of Agriculture, Western Australia

December 2004

Department of Agriculture
Government of Western Australia

HAL
Know-how for Horticulture™

HORTICULTURE AUSTRALIA PROJECT VG03057

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This is the final report for Project VG03057 'Scoping study on the importance of virus diseases in Australian vegetable cucurbit crops'.

December 2004

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MEDIA SUMMARY

Virus diseases cause serious losses in yield and quality in field grown vegetable cucurbit crops worldwide. In Australia, five viruses were known to infect cucurbits but the distribution and incidence of these viruses in Northern Australian crops needed to be determined.

During 2003/04, a survey was done to determine the incidence and distribution of these virus diseases in vegetable cucurbit crops growing in Western Australia (Broome, Carnarvon, Kununurra and Perth), the Northern Territory (Darwin and Katherine) and Queensland (Ayr, Bundaberg, Clare, Giru, Mareeba, Rockhampton and Wowan). The viruses tested for were *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-cucurbit strain* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Some crops were also tested for luteoviruses. Melons were included as these crops constitute a reservoir of infection for vegetable cucurbit crops, and such crops had not been surveyed before in WA and NT.

Widespread infection with viruses was found in vegetable cucurbit crops in WA, NT and Qld resulting not only in damaging yield losses but also in serious fruit quality downgrades and rejections.

Survey in Western Australia (WA) and Northern Territory (NT)

Overall, in WA and NT, 43 vegetable cucurbit growing farms and 172 crops were sampled. From each crop, shoot samples were collected from plants chosen at random and from symptomatic plants. Shoots samples were sometimes also collected from potential alternative virus hosts (cucurbit volunteer plants and weeds).

Overall, 72% of farms and 56% of crops sampled were virus-infected. The growing areas worst affected by virus diseases were Darwin and Carnarvon, and least affected were Katherine and Perth. For WA, overall 78% farms and 56% of crops were virus-infected, while in NT the corresponding figures were 55% of farms and 54% of crops. Overall virus incidences in individual crops sometimes reached 100% infection. Crops of cucumber, melon, pumpkin, squash and zucchini were all infected, with squash and zucchini being the most severely damaged.

The most widespread viruses were ZYMV and PRSV, each being detected in 5 and 4 out of 6 cucurbit growing areas respectively with infected crop incidences of <1-100%. SqMV was detected in 2 cucurbit growing areas. WMV and CMV were found in 3 and 4 out of 6 cucurbit growing areas respectively, but generally at low incidences in infected crops (<1-8%). Infection with luteovirus was found in 3 growing areas but only occurred occasionally.

Infection of individual crops by more than one virus was common with up to 4 viruses found within the same crop. Virus-resistant pumpkin cultivars had little infection when adjacent virus-susceptible cucurbit crops were heavily virus-infected. Viruses were

detected in cucurbit volunteer plants and weeds indicating that they act as important reservoirs for spread to nearby cucurbit crops

Survey in Queensland (Qld)

Crops of the vegetable cucurbits pumpkin, squash and zucchini were surveyed for virus infection in 7 locations in the 3 major cucurbit growing areas of Queensland (Qld), north (Ayr, Clare, Giru, Mareeba), central (Rockhampton, Wowan) and south (Bundaberg). Virus-infection was found in 17/20 farms and 78% of crops, and virus-infected crops often suffered severe yield reduction and high fruit rejection rates. In north Qld, 8/10 farms and 9/12 crops were virus-infected. In central Qld, 4 of 6 farms surveyed had virus present in 7/10 crops. In south Qld, each of the 6 crops surveyed on 5 farms were virus-infected.

Papaya ringspot virus-cucurbit strain (PRSV) was the virus most frequently detected, occurring in every crop found virus-infected regardless of cucurbit type or location in the state, with individual crop incidences of up to 100% in mature crops. *Zucchini yellow mosaic virus* (ZYMV) was found in crops in the Ayr, Clare and Giru areas of north Qld with infection levels of up to 100% in individual crops.

Crops co-infected with PRSV and ZYMV were often found in north Qld. However, PRSV was the only virus found in pumpkin crops in central Qld (Wowan and Rockhampton) and in the Mareeba area of north Qld. A low incidence of *Watermelon mosaic virus* (WMV) was found at Giru in north Qld and Bundaberg in south Qld. Neither *Cucumber mosaic virus* (CMV) nor *Squash mosaic virus* (SqMV) were detected in any vegetable cucurbit types sampled during the survey but CMV was detected in bitter melon at Bundaberg.

General conclusions (WA, NT and Qld)

In general, established vegetable cucurbit growing farms in close proximity to others and with poor crop hygiene suffered badly from virus epidemics, while isolated farms with large-sized, crops or that had only recently started growing cucurbits had less infection.

The extent of infection revealed in vegetable cucurbit crops in this survey, and the resulting financial losses to growers from virus-induced yield losses and high fruit downgrades and rejection rates, is cause for concern for the Australian vegetable cucurbit industry.

TECHNICAL SUMMARY

Virus diseases cause serious losses in yield and quality in field grown vegetable cucurbit crops worldwide. In Australia, five viruses were known to infect cucurbits but the distribution and incidence of these viruses in Northern Australia needed to be determined. During 2003/04, a survey was done to determine the incidence and distribution of these virus diseases in vegetable cucurbit crops growing in Western Australia (Broome, Carnarvon, Kununurra and Perth), the Northern Territory (Darwin and Katherine) and Queensland (Ayr, Bundaberg, Clare, Giru, Mareeba, Rockhampton and Wowan). The viruses tested for were *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-cucurbit strain* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Some crops were also tested for luteoviruses. Melons were included as these crops consititute a reservoir of infection for vegetable cucurbit crops, and such crops had not been surveyed before in WA and NT.

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TECHNOLOGY TRANSFER

Refereed papers

Coutts B.A. and Jones R.A.C (submitted). Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. *Australian Journal of Agricultural Research*

Conference Abstracts

Coutts B.A. and Jones R.A.C (2004). Incidence and distribution of virus diseases in cucurbit crops in the Northern Territory and north-western Australia. In *Proceedings of the 6th Australasian Plant Virology Workshop*, Gold Coast, Queensland, 1-2 September, 2004, p66.

Extension /Advisory Notices

Coutts B (2004) Virus diseases in cucurbit crops in Northern Australia. *WA Grower* **36**, p.29

Persley D. (2004) Virus disease in cucurbits and survey results. *Crop Technical Newsletter*. Bundaberg**.**

Jones R (2003) Virus found in cucurbit study. *Good Fruit and Vegetables*. 14 (5), p. 27

Workshops/Grower meeting

Vegetable growers meeting on virus diseases of cucurbits and summary of survey results at Kununurra, Western Australia. October 2004

Vegetable growers meeting on virus diseases in cucurbits at Bundaberg, Queensland. May 2004

Vegetable growers meeting at Virginia, South Australia. National Vegetable Pathologist Woking Group. April 2004

FINANCIAL ANALYSIS OF THE PROJECT

HAL Funding Budget vs Actuals

Life of Project 2003/04

Funding received	**Budget**	**Actual**	**Variance**
2003/04	$67 000.00	$67 000.00	$0.00
Totals	**$67 000.00**	**$67 000.00**	**$0.00**
Expenditure Actuals	**Operating**	**Capital**	**Total**
2003/04	$67 000.00	$0.00	$67 000.00
Totals	**$67 000.00**	**$0.00**	**$67 000.00**

SECTION 1.0

Incidence and distribution of viruses infecting cucurbit crops in the NorthernTerritory and Western Australia

(draft of paper submitted to *Australian Journal of Agricultural Research*)

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Note: Crops of watermelon, rockmelon and honeydew melon were included in this survey as they are hosts for the viruses that infect vegetable cucurbit crops and constitute a key potential virus reservoir for spread of infection to vegetable cucurbits. Also, they were often grown in close proximity to vegetable cucurbit crops.

Abstract. During 2003/04, a survey was done to determine the incidence and distribution of virus diseases in cucurbit crops growing at Kununurra, Broome, and Carnarvon in north-western Australia, Perth in south-western Australia, and Darwin and Katherine in the Northern Territory**.** Overall, 43 cucurbit growing farms and 172 crops were sampled. From each crop, shoot samples were collected from plants chosen at random and from symptomatic plants. Shoots samples were sometimes also collected from potential alternative virus hosts (cucurbit volunteer plants and weeds). All samples were tested by ELISA using antibodies to *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-cucurbit strain* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Samples from one third of the crops were also tested by TBIA using generic luteovirus antibodies. Overall, 72% of farms and 56% of crops sampled were virus-infected. The growing areas worst affected by virus diseases were Darwin and Carnarvon, and least affected were Katherine and Perth. For WA, overall 78% farms and 56% of crops were virus-infected, while in NT the corresponding figures were 55% of farms and 54% of crops. Overall virus incidences in individual crops sometimes reached 100% infection. Crops of cucumber, melon, pumpkin, squash and zucchini were all infected, with squash and zucchini being the most severely damaged. The most widespread viruses were ZYMV and PRSV, each being detected in 5 and 4 out of 6 cucurbit growing areas respectively with infected crop incidences of <1-100%. SqMV was detected in 2 cucurbit growing areas. WMV and CMV were found in 3 and 4 out of 6 cucurbit growing areas respectively, but generally at low incidences in infected crops (<1-8%). Infection with luteovirus was found in 3 growing areas but only occurred occasionally. Infection of individual crops by more than one virus was common and up to 4 viruses found within the same crop. Virus-resistant pumpkin cultivars had little infection when adjacent virus-susceptible cucurbit crops were heavily virus-infected. Viruses were detected in cucurbit volunteer plants and weeds indicating that they act as important reservoirs for spread to nearby cucurbit crops. In

general, established cucurbit growing farms in close proximity to others and with poor crop hygiene suffered badly from virus epidemics, while isolated farms with large-sized, crops or that had only recently started growing cucurbits had less infection. The extent of infection revealed in this survey, and the resulting financial losses to growers from virus-induced yield losses and high fruit rejection rates, is cause for concern for the Australian cucurbit industry.

Additional keywords: *Cucumber mosaic virus, Papaya ringspot virus-cucurbit strain, Squash mosaic virus, Watermelon mosaic virus, Zucchini yellow mosaic virus*, luteoviruses, mixed infection, virus disease, alternative hosts, losses, economic impact, control.

Introduction

The value of vegetable cucurbit and melon production in the Northern Territory (NT) and Western Australia (WA) is over $40 million annually (Anonymous 2003). Outside Queensland (Qld), the primary cucurbit growing areas in Northern Australia are Kununurra, Broome, Carnarvon, Darwin and Katherine. The cucurbit industry supplies both domestic and export markets (Sherrard 2003). The principal vegetable cucurbit crops grown are the 'green slicing' and 'Lebanese' types of cucumber (*Cucumis sativus*), zucchini and squash (*Cucurbita pepo*), Jarrahdale pumpkin (*Cucurbita maxima*), and butternut and Japanese pumpkin (*Cucurbita moschata*). The melons grown are predominantly watermelon (*Citrullus lanatus*), rockmelon and honeydew melon (*Cucumis melo*). In addition, several exotic Asian vegetable cucurbit crops are produced in small amounts.

The principal viruses infecting cucurbit crops within Australia are *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-cucurbit strain* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) (Greber 1969, 1978; McLean and Price 1984; Greber *et al*. 1987; Buchen-Osmond *et al*. 1988). All of these viruses cause severe diseases in cucurbit crops, resulting in substantial yield and quality losses (Thomas 1971; McLean *et al*. 1982; Herrington 1987; Greber *et al*. 1988; Fletcher *et al*. 2000). They are also important overseas, along with *Cucurbit aphid-borne yellows virus* (CABYV), a luteovirus not so far found in Australia that causes heavy yield losses in cucurbit crops in France, Spain, Morocco, and Lebanon (Lecoq 1992; Abou-Jawdah 2000). The natural host range of another luteovirus in Australia, *Beet western yellows virus* (BWYV), includes cucurbits (Buchen-Osmond *et al*. 1988). CMV, PRSV, WMV and ZYMV are all non-persistently aphid-transmitted viruses, with *Myzus persicae* (green peach aphid) and *Aphis gossypii* (melon aphid) (Greber 1969; Greber *et al*. 1988) being the principal vectors in cucurbit crops. CABYV and BWYV are persistently aphid-transmitted and, again, *M. persicae* and *A. gossypii* are important vectors for them (Lecoq *et al*. 1992). SqMV is transmitted by the '28-spotted ladybird' in Australia (*Henosepilachna vigintioctopunctata*) (Greber 1969). It is also readily seed-borne in cucurbits (Thomas 1973), while seed transmission of ZYMV and WMV may occur occasionally at very low levels in cucurbits (Fletcher *et al*. 2000). SqMV is stable and reaches high concentrations in the plant and contact transmission is another method by which it spreads through infected crops and between nearby farms.

(Fletcher and Herman 2000). This occurs by movement of machinery (eg. spray equipment) and personnel (weeding and cutting).

PRSV, SqMV, WMV and ZYMV naturally infect certain non-cucurbitaceous weed hosts as well as certain wild cucurbit species that are often present in Australian cucurbit growing areas where they act as virus sources (Greber 1969; Buchen-Osmond *et al*. 1988; Fletcher *et al*. 2003; Horlock and Persley 2001). CMV has a wide natural host range infecting more than 40 plant families including several weed and crop species which act as virus reservoir hosts in Australia (Francki *et al*. 1979; Jones 1988). The natural host range of CABYV is limited to cucurbitaceous species overseas (Lecoq *et al*. 1992), while BWYV has a broad host range infecting more than 23 plant families including several Australian weeds and crops that serve as reservoir hosts for susceptible crops (Buchen-Osmond *et al*. 1988; Coutts and Jones 2000).

In WA, the viruses recorded infecting cucurbit crops are CMV and ZYMV at Carnarvon (McLean and Price 1984; Greber *et al*. 1987), ZYMV, PRSV and SqMV at Kununurra (Jones 1996), and CMV at Perth (McLean and Price 1984). In NT, PRSV, ZYMV and SqMV are recorded infecting cucurbit crops in the Darwin area (Conde 2004). This paper reports the results of surveys to determine the incidence and distribution of five viruses infecting cucurbit crops growing in the 6 major cucurbit producing areas in Western Australia and the Northern Territory. One third of survey samples were also tested for infection with luteoviruses using generic luteovirus antibodies. Samples from potential alternative hosts were sometimes also tested for infection with the same viruses.

Materials and Methods

Glasshouse grown plants and inoculations

All plants were grown in insect-proof, air-conditioned glasshouses maintained at 15-20^{o}C. Plants of canola (*Brassica napus*) cv. Pinnacle, cucumber cv. Pronto, squash cv. Green Buttons, zucchini cv. Blackjack, and subterranean clover (*Trifolium subterraneum*) cv. Woogenellup were grown in a steam-sterilized soil, sand and peat mix (1:1:1). For sap inoculations, infected leaves were ground in 0.1M phosphate buffer, pH 7.2, and the sap mixed with 'celite' before being rubbed onto leaves of the plants to be inoculated. For aphid inoculations, *M. persicae* were starved for 2 h, then placed on infected leaves for 2 days prior to feeding on healthy plants (10 aphids/plant) for 1-2 days before being killed with aphicide.

Virus isolates and antisera

Isolates used initially were ZYMV-Qld1, SqMV-375 and PRSV-Qld1 obtained from Denis Persley, QDPI, Qld. Subsequently, isolates ZYMV-Can1 from Carnarvon, SqMV-Kun1 from Kununurra, and PRSV-Brm1 from Broome were used instead. These virus isolates were maintained by sap inoculation to plants of cucumber, squash and zucchini. CMV isolate SN (Jones 1988) was cultured by sap inoculation to plants of subterranean clover. These virus cultures and freeze-dried sap containing WMV obtained from Loewe Biochemica, Germany were used as positive controls in enzyme-linked immunosorbent assay (ELISA). BWYV isolate WA-1 came from previous work (Coutts and Jones

2000). The BWYV culture was maintained by aphid inoculation to plants of canola and used as positive control in tissue blot immunosorbent assay (TBIA).

Polyclonal antisera to ZYMV, PRSV, SqMV, CMV and WMV were obtained from Loewe Biochemica, Germany, generic monoclonal antibodies specific to luteoviruses from DSMZ, Germany, and polyclonal antiserum to BWYV from BioRad, France.

Enzyme-linked immunosorbent assay
Leaves from shoot samples of cucumber, melon, pumpkin, squash and zucchini were extracted (1g/20ml) in phosphate buffered saline (10mM potassium phosphate, 150mM sodium chloride), pH 7.4, containing 5ml/L Tween 20 and 20g/L polyvinyl pyrrolidone, using a leaf press (Pollahne, Germany). The sample extracts were tested for infection by double antibody sandwich ELISA as described by Clark and Adams (1977). Each sample was tested in duplicate wells in microtitre plates and appropriate infected and healthy leaf samples were included in paired wells as controls. The substrate used was 0.6mg/mL of *p*-nitrophenyl phosphate in 100ml/L of diethanolamine, pH 9.8. Absorbance values (A_{405}) were measured in a Multiskan plate reader (Labsystems, Finland). Positive absorbance values were always at least 10 times those of healthy sap.

Tissue blot immunoassay (TBIA)
Cucumber, melon, pumpkin, squash and zucchini shoot samples were tested singly or bound in bundles of up to 10 with parafilm. A scapel was used to cut the ends off single shoot or shoot bundle samples and the cut surfaces then pressed twice onto 0.45µm pore size nitrocellulose membrane (Schleicher and Schuell, Inc., Keene, New Hampshire, U.S.A.). The procedure for TBIA was as described by Coutts and Jones (2000) and Latham and Jones (2001). Tissue prints were blocked in phosphate buffered saline containing 40g/L BSA for 1 hour. They were incubated with luteovirus-specific primary antibodies and then with enzyme-labelled species specific (goat anti-mouse) secondary antibodies conjugated to alkaline phosphatase (Southern Biotechnology Associates, USA) each for 2 hours. The substrate solution contained 14mg nitroblue tetrazolium and 7mg 5-bromo-chloro-3-indolyl phosphate in 40mL of substrate buffer consisting of 0.1M Tris, 0.1M NaCl, 5mM $MgCl_2$, pH 9.5. Development of purple colour in the phloem was observed using a binocular microscope and revealed luteovirus presence in tissue prints from infected samples. Some positive luteovirus samples were retested in the same way using BWYV-specific primary antibodies and then with enzyme-labelled species specific (goat anti-rabbit) secondary antibodies conjugated to alkaline phosphatase.

Field surveys
The crops surveyed consisted of cucumber (green slicer and Lebanese types), melon (honeydew melon, rockmelon and watermelon), pumpkin (butternut, Japanese and Jarrahdale types), squash and zucchini growing in the 4 principal cucurbit growing areas in WA and 2 main growing areas in NT (Fig. 1). In WA, a total of 9 farms (38 crops) at Kununurra and 6 farms (39 crops) at Broome were surveyed in August 2003 and July 2004; 9 farms (33 crops) at Carnarvon in October 2003; and 8 farms (25 crops) at Perth in April and November 2004. In NT, 3 farms (20 crops) at Darwin and 8 farms (22 crops) at Katherine were surveyed in September 2003. For each crop, normally 100

young shoots were sampled (one/ plant) at random, every five paces along a diagonal or in Z or W shaped patterns within each crop. Where there were insufficient plants (or most plants were symptomatic) fewer random samples were sometimes collected but always >25/crop. In addition, for each crop with symptomatic plants, shoots from 1-3 plants with representative virus-like symptoms were collected separately and kept apart from others. All samples were placed in polyethylene bags, and the bags were then labeled, cooled using ice or freezer packs, sealed, and transported in cooler boxes with freezer packs to the laboratory in Perth. To determine virus incidence, the random samples were tested by ELISA, initially in groups of 5 or 10 leaves each from different shoots or, where most were symptomatic, individually. When necessary, grouped samples were retested at lower levels of grouping or singly. Percentage incidence from grouped sample results was estimated using the formula of Gibbs and Gower (1960). Symptomatic samples were always tested individually. One third of samples were also tested by TBIA using generic luteovirus antibodies in bundles of 10 shoots (random shoots) or individually (symptomatic samples). In a few instances, samples testing positive using luteovirus antibodies were retested by TBIA using BWYV-specific antibodies.

In some instances, shoots were collected from volunteer and wild (weed) cucurbit plants growing within or adjacent to cucurbit crops. Both random and potentially symptomatic shoot samples were collected. These samples were bagged, cooled, transported and tested by ELISA and TBIA as described above for cucurbit crop samples.

Results

Overall virus occurrence

In WA and NT, a total of 43 cucurbit growing farms and 172 cucurbit crops were surveyed in 2003/04. Overall, 72% of farms and 56% of crops were found to be virus infected (Table 1). For WA, overall 78% farms and 56% of crops were virus-infected, while in NT the corresponding figures were 55% of farms and 54% of crops. For the individual growing areas in WA, all farms surveyed at Broome-1, Carnarvon and Kununurra were virus-infected with 90%, 80% and 71% of crops infected respectively in these areas. In contrast, at Broome-2 and Perth less than one third of farms within each area and 5% and 16% of crops respectively were infected. In NT, at Darwin all farms and crops sampled were infected but at Katherine the corresponding virus infection figures were only 37% of farms and 14% of crops.

When the data for established farms was separated from those for new or isolated farms, 100% of the former, but only 37% of the latter were virus-infected (Table 1). The percentages of established cucurbit growing farms and crops infected respectively were: Broome-1, 100% (farms), 90% (crops); Carnarvon, 100%, 80%; Kununurra, 100%, 71%; and Darwin, 100%, 100%. For the newly established cucurbit growing areas of Broome-2 and Katherine and the isolated cucurbit growing farms at Perth 33%, 37%, and 38% of farms, and 5%, 14% and 16% of crops respectively were virus-infected. Although incidences of infection within individual crops sometimes reached 100% on established farms in Broome-1, Carnarvon, Darwin and Kununurra, they were never more than 8% at Broome-2, Katherine and Perth. In Perth, 3 additional farms (10 crops) growing

cucumbers in glasshouses were also sampled, but no virus was detected in random samples from any of their crops.

Occurrence of individual viruses

Infection with ZYMV was detected in all locations sampled except Broome, and, overall, 46% of farms and 35% of crops were infected with it (Table 2). All infected crops at Carnarvon had ZYMV infection, while in Darwin and Kununurra 72% and 74% of virus-infected crops respectively were infected with this virus. At Katherine and Perth, ZYMV was found in 1 of 3 and 2 of 4 virus-infected crops. PRSV was detected at Broome-1, Darwin, Katherine and Kununurra, and overall 23% of farms and 17% of crops were infected with it. PRSV was most prevalent at Broome-1, where it was detected in all virus-infected crops but one. At Darwin, Kununurra and Katherine 39%, 11% and 2 of 3 virus-infected crops had PRSV infection respectively. Overall, SqMV was detected in 21% of farms and 10% of all crops surveyed. However, in 2003 it was only found at Kununurra where it was very common occurring in 63% of virus-infected crops. In 2004 it was detected at one farm at Broome-1. CMV was detected in cucurbit crops at Broome-1, Carnarvon, Darwin and Perth but only in 3% of overall crops. WMV was found in cucurbit crops at Carnarvon, Kununurra and Perth, being detected in only 2% of crops overall.

Infection of different types of cucurbits

In WA and NT together, the overall percentages of crops found virus-infected were:- cucumber-61%, melon-42%, pumpkin-61%, squash-70%, and zucchini-63% (Table 3). However, overall virus occurrence tended to be under-estimated in zucchini and squash crops due to heavy roguing out of visibly symptomatic plants by growers. All of the different types of cucurbits became infected with ZYMV, PRSV and SqMV. ZYMV was detected in cucumber, pumpkin, squash and zucchini with 42%, 44%, 60% and 36% of crops infected at incidences within individual infected crops of <1-100%, <1-90%, <1-75% and <1-90% respectively. It also infected 20% of melon crops with individual infected crop incidences of <1-70%. PRSV was found in 15% of cucumber, 20% of melon, 16% of pumpkin, 10% of squash and 18% of zucchini crops. Individual infected crop incidences in pumpkin and zucchini were <1-100%, while those in cucumber, melon and squash were 44-57%, 1-53% and 7% respectively. SqMV was detected in 7% of melon, 8% of cucumber and 11% of pumpkin crops at individual infected crop incidences of 1-4%, 3-60% and <1-56% respectively. In squash and zucchini, the corresponding figures were 20% and 18% of crops at individual infected crop incidences of 12-18% and 5-53% respectively. CMV was found infecting 2%, 5% and 5% of melon, pumpkin and zucchini crops respectively at individual infected crop incidences of <1-8%. It was not detected in cucumber or squash. WMV was detected in 10%, 5% and 4% of squash, zucchini and pumpkin crops respectively, at individual infected crop incidences of <1-2%, but was not detected in melon or cucumber.

Multiple virus infections

Of the 172 crops sampled, 20 had multiple infections with different viruses (Table 3). For example, at Kununurra 4 viruses (ZYMV, SqMV, PRSV and WMV) were detected in shoot samples collected from the same Jarrahdale pumpkin crop, and 3 viruses (ZYMV,

SqMV and PRSV) in a single watermelon crop. Two viruses were detected in each of 18 crops. For example, 9 of those at Kununurra including cucumber, melon, pumpkin, squash and zucchini were co-infected with ZYMV and SqMV. Other combinations included: ZYMV and CMV together in 3 crops (melon and pumpkin) from Carnarvon; PRSV and CMV from one pumpkin crop each at Darwin and Broome; and ZYMV and WMV from one crop each from Carnarvon (pumpkin) and Kununurra (squash). The combinations of PRSV and ZYMV, and PRSV and SqMV were detected once each, in Darwin with the former (pumpkin) and at Broome (zucchini) with the latter.

Incidence of luteoviruses

Of the 172 cucurbit crops surveyed in WA and NT, samples from 58 of them were also tested for luteovirus infection. Luteovirus infection was detected in 9 of these crops in 3 locations (Table 4). In Perth, 3/3 luteovirus-infected crops of cucumber on one farm had incidences of 10-49%. In Carnavon, 3/5 pumpkin crops on two farms had luteovirus incidences of 21-48%. In Kununurra, 2 pumpkin and one melon crop, one each from 3 farms were infected at incidences of 1-2%. No luteovirus infection was detected in samples from cucurbit crops from Broome, Darwin or Katherine. Samples from 36 crops were retested for BWYV infection and the luteovirus in one melon crop at Kununurra was identified as BWYV. These results suggest presence of an as yet unidentified luteovirus(es) infecting Australian cucurbit crops.

Infection in virus-resistant cultivars

Three crops each of virus-resistant Jarrahdale pumpkin cv. Dulong and butternut pumpkin cv. Sunset were sampled, both cultivars being resistant to infection with PRSV, WMV and ZYMV (Anonymous 1999, 2001). In Darwin, one farm grew one crop each of cvv. Dulong and Sunset, but no symptomatic plants were seen and no virus was detected in either crop when the surrounding cucumber, pumpkin, squash and zucchini crops had ZYMV incidences of up to 70%. In Carnarvon, 2 crops of cv. Dulong were sampled at one farm. One of these crops had no symptomatic plants with no virus detected in random samples despite the surrounding Japanese and butternut pumpkin crops having ZYMV incidences of up to 80%. In the other crop, two symptomatic shoot samples from plants with small axillary shoots with mild leaf mottle were collected in addition to 100 random shoots. ZYMV was detected in the symptomatic plants, but no CMV, PRSV, SqMV, WMV or ZYMV was detected in the random sample, although 25% of them had luteovirus infection. In Kununurra and Katherine, one crop of cv. Sunset was sampled in each location, but no symptomatic plants were seen or virus detected. The surrounding crops of Japanese and Jarrahdale pumpkin had low levels of SqMV (1%) at Kununurra but were healthy at Katherine.

Additional virus(es)

Visual observation of the percentages of symptomatic plants in field cucurbit crops sometimes indicated higher levels of virus infection than were borne out subsequently by the serological tests. For example, at Kununurra, a farm growing cucumber and squash had crops with 100% of plants that were symptomatic showing typical leaf mottle, leaf distortion and plant stunting, but, when tested by ELISA using antibodies to ZYMV, PRSV, WMV, SqMV and CMV, virus was detected in less than 5% of cucumber samples

and less than 20% of squash samples. Similarly at Broome, a mature pumpkin crop had 100% of plants that were symptomatic but when leaves were tested by ELISA less than 5% of plants tested positive for PRSV while CMV, SqMV, WMV and ZYMV were not detected. Such results indicate potentially widespread occurrence of another virus(es) causing similar symptoms in cucurbit crops. Luteovirus infection was not the cause as it was rarely detected in such crops.

Infection of alternative hosts

Random and symptomatic samples were collected from volunteer watermelon plants from cucurbit growing farms at Broome-2, Kununurra, and Perth. Among the 20 and 50 random samples collected from one farm each at Broome-2 and Perth no virus was detected, but WMV was found in 1/7 symptomatic samples collected at Broome-2. At Kununurra, when 15 samples were collected at random, 7 of these were found infected with luteovirus and 8 with ZYMV, while symptomatic samples collected at the same site had 3/5 and 4/4 samples infected with luteovirus and ZYMV respectively. When two symptomatic plants were collected at Carnarvon, both of these were found infected with ZYMV.

Cucurbitaceous weeds were collected from two farms**.** From one farm at Broome-2, WMV was detected in Afghan melons (*Citrullus lanatus*): 7% of 60 randomly collected samples and 2/11 symptomatic samples were WMV-infected. No virus was detected in 4 symptomatic wild melon (*Cucumis myriocarpus*) plants collected from the same farm at Broome-2. In Carnarvon, 2/10 wild melon plants collected at random had luteovirus infection.

The detection failures in symptomatic plants of volunteer watermelon and cucurbitaceous weeds again suggest presence of another virus or viruses not detected by the virus antibodies used in our tests.

Discussion

Widespread infection with viruses was found in cucurbit crops in WA and NT, resulting not only in damaging yield losses but also in serious fruit quality downgrades and rejections. Infected crops were often co-infected with more than one virus, usually with 2 but less often with 3 or even 4 different viruses. All vegetable cucurbit types were diseased, with 70% of squash, 64% of zucchini, 61% of cucumber and 61% of pumpkin crops virus-infected overall. Melon crops were also diseased, 42% being virus-infected. In the established cucurbit growing areas of Broome-1, Carnarvon, Darwin and Kununurra, where cucurbit growing farms and their crops are often in close proximity to each other, virus infection was found on all farms surveyed, with 75% of crops infected and individual crop incidences of up to 100%. In contrast, in the new production areas of Broome-2 and Katherine, or the older growing area with isolated farms at Perth, only 37% of farms and 12% of crops were found infected, and virus incidences within individual infected crops never exceeded 8%.

When interpreting the data we collected, it needs to be stressed that the incidences of virus infection found in this work represent a ‘snapshot’ of levels detected at time of

sampling. Further virus spread occurred after sampling, especially in younger crops. Thus, final virus infection incidences would often have been considerably higher than those found. For example, the survey in Perth was done at 2 different times, initially in April 2004 when the majority of crops had been harvested and later in November 2004 when most were still young. At the latter time, the crops were the first for the season with the first fruit harvest only just beginning: virus was only detected in one of the 11 crops sampled (1% incidence of WMV). Thus, overall virus incidence in the Perth area would probably have been greater if sampling had been done in the main summer production period. Moreover, virus incidences in squash and zucchini crops in the different production areas in WA and NT were considerably underestimated due to roguing. In all areas except Perth, cucurbit growers rigorously rogued out any plants that had virus-like symptoms, often doing this daily to keep on top of the problem. Many growers had given up growing these 2 crops because of the labour intensive rouging required.

ZYMV was the virus most often found, being present on 46% of farms and in 34% of crops overall. PRSV was the next commonest being detected on 23% of farms and 17% of crops. The incidences of these 2 viruses within individual infected crops often reached 100%. SqMV was detected in 21% of farms and 10% of crops overall. CMV and WMV were less frequently present, infection being detected in only 11% and 9% of farms and 3% and 2% of crops respectively. As mentioned in the Introduction, an earlier survey at Kununurra found symptomatic pumpkin and melon infected with ZYMV, PRSV and SqMV (Jones 1996). In 2003/04, we found these same three viruses at Kununurra and, in addition, a low incidence of WMV. McLean and Price (1984) reported CMV and ZYMV infecting cucumber, melon and pumpkin at Carnarvon, and CMV infecting cucumber and pumpkin at Perth. In 2003, ZYMV was found at both locations with CMV and WMV also detected at low levels. We confirmed the earlier report of PRSV and ZYMV in NT cucurbit crops (Conde 2004), but also found CMV in a pumpkin crop at Darwin. As PRSV was confined to the tropical areas of Broome, Kununurra and Darwin, this suggests its adaption to hot growing conditions. In 2003, SqMV was only found in cucurbit crops at Kununurra, but additional sampling in 2004 also detected it at Broome-1 on a farm where it was absent in 2003. As mentioned in the Introduction, SqMV is seed-borne in cucurbits (Greber 1969; Thomas 1973). At Kununurra, cucurbit seed is generally imported from the USA, where SqMV occurs widely (Grogan *et al*. 1959). Possibly, a similar source of imported seed may have introduced the virus to Broome-1 in 2004. In Kununurra, where SqMV was frequently found, large numbers of its '28 spotted ladybird' vector were present on infected crops as well as high numbers of pumpkin beetle (*Aulacophora hilaris*). Pumpkin beetle is not reported to be a SqMV vector. However, since it is often associated with crops with high incidences of SqMV, tests are needed to determine if it can transmit the virus. As regards likely vectors of the aphid-borne viruses found in the survey, the only species commonly found colonising cucurbits was the melon aphid.

The alternative virus reservoir hosts we identified were plants of Afghan melon, wild melon and volunteer watermelon. In cucurbit production areas in WA and NT, there is normally a break when no cucurbit crops are present between successive growing

seasons. For example, at Kununurra, production is restricted to May to October whereas in Perth it is from November to April. Without continuous 'all-year-round' production, viruses that are not readily seed-borne in cucurbits (e.g. CMV, PRSV, WMV and ZYMV) need to infect alternative hosts if they are to persist in-between these growing seasons. At the beginning of the following growing season, their aphid vectors can then spread them into newly sown cucurbit crops. Infected cucurbit weeds, volunteer cucurbit plants and non-cucurbitaceous weeds therefore provide a 'green bridge' of susceptible hosts between growing seasons in WA and NT, as previously reported in Qld (Greber 1969; Persley and Horlock 2003).

Additional virus(es) were almost certainly present infecting cucurbit crops and cucurbitaceous weeds in the survey. Firstly, the typical mosaic, leaf deformation and stunting symptoms found at time of sampling of cucurbit crop and weed plants were not always associated with infection with the viruses we tested for. Secondly, luteovirus infection was also sometimes detected but its presence was not associated with these types of symptoms. Moreover, only on one occasion was the luteovirus concerned identified as BWYV. This suggests that another luteovirus(es) is present. Since its initial identification in 1992, CABYV was found in many European countries and its yellowing symptoms in older leaves can easily be overlooked if plants are already infected by viruses causing mosaic symptoms (Lecoq *et al*. 1992). However, whether the additional luteovirus(es) infecting cucurbits in Australia is CABYV or another luteovirus is not known. These results suggest that at least 2 unidentified viruses are often present in cucurbit crops in Australia, one causing typical mosaic and associated symptoms and the other reacting with generic luteovirus antibody. Further work is required to identify these viruses. A large number of viruses infect cucurbits overseas many of which are economically important so there are several potential candidates (Provvidentii 1996).

During this survey, a number of factors were identified that favoured virus epidemics and high final virus infection incidences in cucurbit crops. Farms with high virus incidences were often in close proximity to neighbouring farms growing cucurbits; had poor hygiene; did not remove old harvested crops promptly or rogue out symptomatic squash and zucchini plants effectively; tended to have small sized-crops; had successive side-by-side plantings; had volunteer cucurbit plants and cucurbit weeds present; did not use virus-resistant cultivars; and used potentially SqMV infected seed. In contrast, farms with low virus incidences were often in new cucurbit growing areas or were isolated farms with large-sized crops; had good hygiene practices; promptly destroyed finished crops; avoided successive side-by-side plantings; rigorously removed weeds and volunteer cucurbits; and rogued out symptomatic squash and zucchini plants effectively. A few also planted virus-resistant pumpkin cultivars. Management strategies that help diminish virus epidemics in cucurbits should include such approaches. Other options include use of reflective mulch to decrease aphid vector landing rates, and applying mineral oil in combination with aphicide sprays to reduce aphid vector numbers (McLean *et al*. 1982; Pinese *et al*. 1994). However, the practice of spraying insecticides alone to suppress colonising aphid vectors often used on the farms surveyed was ineffective in suppressing virus spread. This is to be expected for non-persistently aphid-borne viruses as insecticides rarely act fast enough to prevent their transmission (e.g. Thackray *et al*.

2000). In conclusion, an integrated approach is necessary to diminish overall virus incidence including: use of healthy seed stocks (SqMV-free); isolation; removing any potential alternative virus reservoirs (weeds, volunteer cucurbit plants, old finished crops) during and between growing seasons; roguing out symptomatic plants; using virus-resistant cultivars when available; and restricting movement and handling of plants within crops to minimize SqMV spread.

The extent of virus infection revealed in this survey, and the resulting financial losses to cucurbit growers from virus-induced yield depression and high fruit downgrading and rejection rates, is cause for concern for the Australian national cucurbit industry. Most cucurbit growers consulted during the survey were aware, at least to some extent, of virus infection in their crops and the damage they cause but in many cases were living with the problem rather than attempting to address it in any way apart from using ineffective insecticide applications. Fortunately, as discussed in the previous paragraph, there are simple remedial measures that can be incorporated into the production system to help avoid major losses.

Acknowledgements

We thank L.J. Smith, T. Smith, M. Holland, G. Burchell and A. Grover and for support with collecting and/or testing of samples, L.J. Latham for helpful discussion and cucurbit growers in WA and NT for allowing us to collect samples on their farms. J. Moulden and P. Smith and staff of the WA Department of Agriculture Research Stations at Carnarvon, Kununurra, and B. Conde and S. Bellgard from the NT Department of Business, Industry and Development, helped with locating cucurbit crops. Financial support was provided by Horticulture Australia Limited. Murdoch University provided funded travel to the Kimberley in 2004.

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Figure legend

Fig. 1. Distribution and incidence of virus infection in cucurbit crops in Australia in 2003/04. For each of the main growing areas in WA and NT, overall percentage virus infection values are given for (1) crops found infected and (2) ranges of virus incidence found within individual infected crops.

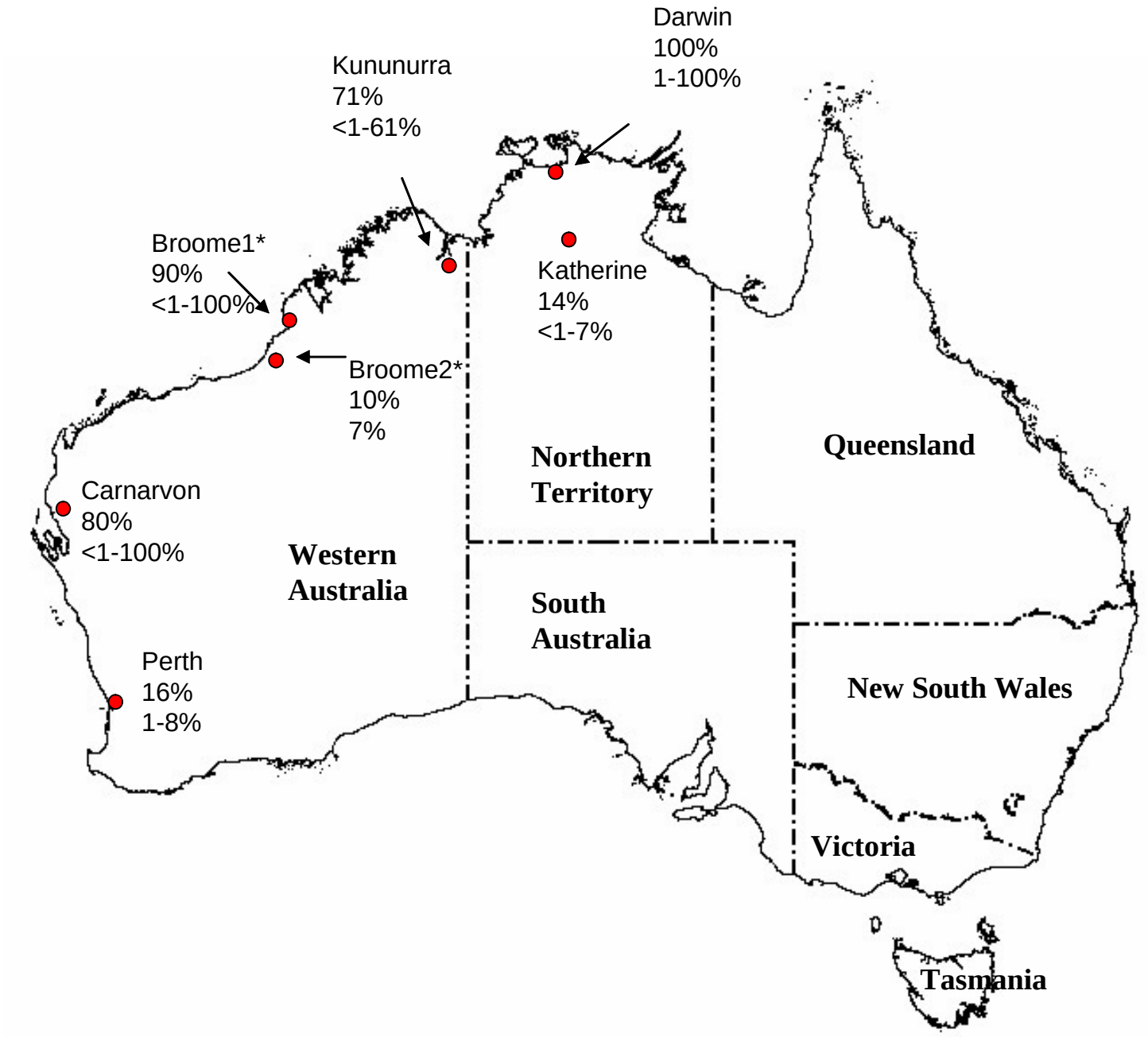
Darwin
100%
1-100%
Kununurra
71%
<1-61%
Broome1*
90%
<1-100%
Katherine
14%
<1-7%
Broome2*
10%
7%
Northern
Territory
Queensland
Carnarvon
80%
<1-100%
Western
Australia
South
Australia
Perth
16%
1-8%
New South Wales
Victoria
Tasmania

Table 1. Incidence of 5 viruses [A] in crops in established versus new or isolated farms in 6 cucurbit growing areas in WA and NT[B]

Location	No. of farms with virus detected/total no. sampled (% farms infected)	No. of crops with virus detected/total no. sampled (% crops infected)	No. of crops with multiple virus infection	No. of crops with > 1% virus incidence	No. of crops with <1% virus incidence	Range of virus incidence (%) within individual infected crops	Viruses detected (no. of crops with each virus)
Established farms							
Broome-1	3/3	18/20 (90)	2	17	1	<1-100	PRSV(17), CMV(1), SqMV(1)
Carnarvon	9/9	25/31 (80)	3	24	1	<1-100	ZYMV(25), CMV(2), WMV(1)
Kununurra	9/9	27/38 (71)	13	21	6	<1-61	ZYMV(20), SqMV(18), PRSV(3), WMV(2)
Darwin	3/3	18/18 (100)	2	18	0	1-100	ZYMV(13), PRSV(7), CMV(1)
TOTAL	**24/24 (100)**	**88/107(75)**	**20**	**80**	**8**		**ZYMV(53), PRSV(27), SqMV(19), CMV(4), WMV(3)**
New or isolated farms							
Broome-2	1/3	1/19 (5)	0	1	0	7	PRSV(1)
Katherine	3/8	3/21 (14)	0	2	1	<1-7	PRSV(2), ZYMV(1)
Perth	3/8	4/25 (16)	0	4	0	1-8	ZYMV(2), CMV(1), WMV(1)
TOTAL	**7/19 (37)**	**8/65 (12)**	**0**	**7**	**1**		**ZYMV(3), PRSV(3), WMV(1), CMV(1)**
COMBINED TOTAL	**31/43 (72)**	**96/172 (56)**	**20**	**87**	**9**	**<1-100**	**ZYMV(56), PRSV(30), SqMV(19), CMV(5), WMV(4)**

[A] CMV, PRSV, SqMV, WMV, ZYMV

[B] Data for virus-resistant cultivars and glasshouse grown crops excluded

Table 2. Incidence of 5 viruses in crops in 6 cucurbit growing areas in WA and NT [A]

Type of crop	No. of farms with virus detected/total no. sampled (% farms infected)	No. of crops with virus detected/total no. sampled (% crops infected)	No. of crops with multiple virus infections	No. of crops >1% virus incidence	No. of crops <1% virus incidence	Range of virus incidence (%) within individual infected crops	Growing area in which virus detected[B] (No. of crops with virus detected)
Zucchini yellow mosaic virus							
Cucumber	6/12	11/26	1	10	1	<1-100	CVN(8), KUN(2), DWN(1)
Melon	7/28	11/57	3	10	1	<1-70	CVN(6), KUN(2), DWN(3)
Pumpkin	11/27	25/57	7	21	4	<1-90	CVN(10), KUN(7), DWN(7), KATH(1)
Squash	4/9	6/10	3	5	1	<1-75	KUN(5), DWN(1)
Zucchini	4/16	8/22	3	7	1	<1-90	CVN(1), KUN(4), PTH(2), DWN(1)
TOTAL	**20/43 (46)**	**61/172 (35)**		**53**	**8**	**<1-100**	**CVN(25), KUN(20), PTH(13), DWN(1), KATH (2)**
Papaya ringspot virus-cucurbit strain							
Cucumber	2/12	4/26	0	4	0	44-57	BME(4)
Melon	6/28	12/57	0	12	0	1-53	BME(6), KATH(2), DWN(4)
Pumpkin	4/27	9/57	4	7	2	<1-100	BME(4), KUN(2), DWN(3)
Squash	1/9	1/10	0	1	0	7	BME(1)
Zucchini	3/16	4/22	2	3	1	<1-100	BME(3), KUN(1)
TOTAL	**10/43 (23)**	**30/172 (17)**		**27**	**3**	**<1-100**	**BME(18), KUN(3), DWN(7), KATH(2)**
Squash mosaic virus							
Cucumber	2/12	2/26	1	2	0	3-60	KUN(2)
Melon	3/28	4/57	3	4	0	1-4	KUN(4)
Pumpkin	4/27	6/57	3	5	1	<1-56	KUN(6)
Squash	2/9	2/10	2	2	0	12-18	KUN(2)
Zucchini	4/16	4/22	3	4	0	5-53	BME(1), KUN(3)
TOTAL	**9/43 (21)**	**18/172 (10)**		**17**	**2**	**<1-60**	**BME(1), KUN(17)**
Cucumber mosaic virus							
Cucumber	0/12	0/26	-	0	0	-	-
Melon	1/28	1/57	1	1	0	8	CVN(1)
Pumpkin	3/27	3/57	3	1	2	<1	BME(1), CVN(1), DWN(1)
Squash	0/9	0/10	-	0	0	-	-
Zucchini	1/16	1/22	0	1	0	8	PTH(1)
TOTAL	**5/43 (11)**	**5/172 (3)**		**3**	**2**	**<1-8**	**BME(1), CVN(2), PTH(1), DWN (1)**
Watermelon mosaic virus							
Cucumber	0/12	0/26	-	0	0	-	-
Melon	0/28	0/57	-	0	0	-	-
Pumpkin	2/27	2/57	2	0	2	<1	CVN(1), KUN(1)
Squash	1/9	1/10	1	1	0	2	KUN(1)
Zucchini	1/16	1/22	-	0	0	1	PTH(1)
TOTAL	**4/43 (9)**	**4/172 (2)**		**1**	**2**	**1-2**	**CVN(1), KUN(2), PTH(1)**

[A] Data from established and new or isolated farms combined, but data from virus-resistant cultivars and glasshouse grown crops excluded

[B] Growing area code: BME = Broome, CVN = Carnarvon, KUN = Kununurra, PTH = Perth, DWN = Darwin, KATH = Katherine

Table 3. Distribution and incidence of 5 viruses[A] in different types of cucurbit crops growing in 6 growing areas in WA and NT[B]

Type of crop	No. of farms with virus detected/total no. sampled (% farms infected)	No. of crops with virus detected/total no. sampled (% crops infected)	No. of crops with multiple virus infections	No. of crops with >1% virus incidence	No. of crops with < 1% virus incidence	Range of virus incidences (%) within individual infected crops	Viruses detected (no. of crops with each virus)
Broome-1 (WA)							
Cucumber	2/2	4/6	0	4	0	28-57	PRSV (4)
Melon	2/2	5/5	0	5	0	7-53	PRSV (5)
Pumpkin	2/2	5/5	1	4	1	<1-64	PRSV (4), CMV (1)
Squash	1/1	1/1	0	1	0	7	PRSV (1)
Zucchini	3/3	3/3	1	3	1	4-100	PRSV(3), SqMV (1)
TOTAL	**3/3**	**18/20 (90)**	**2**	**17**	**1**	**<1-100**	**PRSV (17), CMV (1), SqMV (1)**
Broome-2 (WA)							
Cucumber	0/1	0/3	0	0	0	-	-
Melon	1/3	1/11	0	1	0	7	PRSV (1)
Pumpkin	0/2	0/4	0	0	0	-	-
Squash	0	0	0	0	0	-	-
Zucchini	0/1	0/1	0	0	0	-	-
TOTAL	**1/3**	**1/19 (5)**	**0**	**1**	**0**	**7**	**PRSV (1)**
Carnarvon (WA)							
Cucumber	3/3	8/8	0	8	0	5-100	ZYMV (8)
Melon	5/5	6/9	1	5	1	<1-70	ZYMV (6), CMV (1)
Pumpkin	5/6	10/13	2	10	0	<1-80	ZYMV(10), CMV(1), WMV(1)
Squash	0	-	-	-	-	-	-
Zucchini	1/1	1/1	0	1	0	90	ZYMV (1)
TOTAL	**9/9**	**25/31 (80)**	**3**	**24**	**1**	**<1-100**	**ZYMV (25), CMV (2),WMV (1)**
Darwin (NT)							
Cucumber	1/1	1/1	0	1	0	70	ZYMV (1)
Melon	3/3	6/6	1	6	0	1-50	ZYMV (3), PRSV (4)
Pumpkin	3/3	9/9	2	9	0	1-100	ZYMV(7), PRSV(3), CMV(1)
Squash	1/1	1/1	0	1	0	75	ZYMV (1)
Zucchini	1/1	1/1	0	1	0	33	ZYMV (1)
TOTAL	**3/3**	**18/18 (100)**	**2**	**18**	**0**	**1-100**	**ZYMV (13), PRSV (7), CMV (1)**

Katherine (NT)							
Cucumber	0	-	-	-	-	-	-
Melon	2/7	2/13	0	2	0	1-7	PRSV (2)
Pumpkin	1/5	1/8	0	0	1	<1	ZYMV (1)
Squash	0	-	-	-	-	-	-
Zucchini	0	-	-	-	-	-	-
TOTAL	**3/8**	**3/21 (14)**	**0**	**2**	**1**	**<1-7**	**PRSV (2), ZYMV (1)**
Kununurra (WA)							
Cucumber	3/3	3/4	1	2	1	<1-61	ZYMV (2), SqMV (2)
Melon	3/4	4/5	2	4	0	1-34	ZYMV (2), SqMV (4)
Pumpkin	7/7	10/16	4	7	3	<1-34	ZYMV(7), SqMV(6), PRSV(2), WMV(1)
Squash	4/4	5/7	3	4	1	<1-40	ZYMV(5), SqMV(2),WMV (1)
Zucchini	4/4	5/6	3	4	1	<1-54	ZYMV(4), SqMV(3), PRSV(1)
TOTAL	**9/9**	**27/38 (71)**	**13**	**21**	**6**	**<1-61**	**ZYMV (20), SqMV(17), PRSV(3), WMV(2)**
Perth (WA)							
Cucumber	0/2	0/4	0	0	0	-	-
Melon	0/3	0/8	0	0	0	-	-
Pumpkin	0/2	0/2	0	0	0	-	-
Squash	0/1	0/1	0	0	0	-	
Zucchini	2/5	4/10	0	4	0	1-8	ZYMV (2), CMV (1), WMV (1)
TOTAL	**3/8**	**4/25 (16)**	**0**	**4**	**0**	**1-8**	**ZYMV (2), CMV (1), WMV (1)**
Total (WA and NT)							
Cucumber	9/12	16/26	1	15	1	<1-100	PRSV(4), ZYMV(11), SqMV(2)
Melon	16/28	24/57	3	23	1	<1-70	PRSV(12), ZYMV(11), SqMV(4), CMV(1)
Pumpkin	18/28	35/57	9	30	5	<1-100	PRSV(9), ZYMV(25), SqMV(6), CMV(3), WMV(2)
Squash	6/7	7/10	3	6	1	<1-75	PRSV(1), ZYMV(6), SqMV(2), WMV(1)
Zucchini	12/16	14/22	4	13	1	<1-100	PRSV(4), ZYMV(8), SqMV(4), CMV(1), WMV(1)
TOTAL	**31/43 (72)**	**97/172 (56)**	**20**	**87**	**9**	**<1-100**	**PRSV(30), ZYMV(61), SqMV(18), CMV(5), WMV(4)**

[A] CMV, PRSV, SqMV, WMV, ZYMV

[B] Data from established and new or isolated farms combined, but data for virus-resistant cultivars and glasshouse grown crops excluded

Table 4. Incidence of luteovirus infection in different cucurbit growing areas and crops in WA and NT[A]

	No. of farms with virus detected/total no. sampled (% farms infected)	No. of crops with virus detected/total no. sampled (% crops infected)	Range of virus incidence (%) within individual infected crops
Broome	0/6	0/18	-
Carnarvon	2/9	3/7	21-48
Kununurra	3/9	3/14	1-2
Darwin	0/3	0/9	
Perth	1/4	3/3	10-49
Katherine	0/8	0/10	
Total	**6/39 (15)**	**9/58**	
Cucumber	1/12	3/4	10-49
Pumpkin	4/26	5/13	1-48
Squash	0/6	0/1	-
Zucchini	0/12	0/4	
Melon	1/26	1/36	1
Total	**6/39 (15)**	**9/58**	

[A]Data from established and new or isolated farms combined and virus-resistant cultivars included; data for glasshouse crops excluded

SECTION 2.0

Cucurbit Virus Survey - Queensland 2004

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Summary

Crops of the vegetable cucurbits pumpkin, squash and zucchini were surveyed for virus infection in 7 locations in the 3 major cucurbit growing areas of Queensland (Qld), north (Ayr, Clare, Giru, Mareeba), central (Rockhampton, Wowan) and south (Bundaberg). Virus-infection was found in 17/20 farms and 78% of crops, and virus-infected crops often suffered severe yield reduction and high fruit rejection rates. In north Qld, 8/10 farms and 9/12 crops were virus-infected. In central Qld, 4 of 6 farms surveyed had virus present in 7/10 crops. In south Qld, each of the 6 crops surveyed on 5 farms were virus-infected. *Papaya ringspot virus-cucurbit strain* (PRSV) was the virus most frequently detected, occurring in every crop found virus-infected regardless of cucurbit type or location in the state, with individual crop incidences of up to 100% in mature crops. *Zucchini yellow mosaic virus* (ZYMV) was found in crops in the Giru, Clare and Ayr areas of north Qld with infection levels of up to 100% in individual crops. Crops co-infected with PRSV and ZYMV were often found in north Qld. However, PRSV was the only virus found in pumpkin crops in central Qld (Wowan and Rockhampton) and in the Mareeba area of north Qld. A low incidence of *Watermelon mosaic virus* (WMV) was found at Giru in north Qld and Bundaberg in south Qld. Neither *Cucumber mosaic virus* (CMV) nor *Squash mosaic virus* (SqMV) were detected in any vegetable cucurbit types sampled during the survey but CMV was detected in bitter melon at Bundaberg. The high levels of infection found with PRSV and ZYMV, and the resulting yield losses and high fruit rejection rates, are cause for concern for the Qld vegetable cucurbit industry.

Material and methods

Field surveys of vegetable cucurbit crops
The vegetable cucurbit crops surveyed were growing in the principal cucurbit growing areas in north, central and south Qld. In north Qld (Mareeba, Giru, Clare and Ayr), 9 farms and 12 crops were surveyed while corresponding figures for central Qld (Wowan and Rockhampton) were 6 farms and 10 crops, and for south Qld (Bundaberg) 5 farms and 6 crops. For each crop, an overall visual assessment of the percentage of plants with typical virus symptoms was done. In addition, 10-20 leaves were collected from each crop to identify the predominant virus present. The samples were placed in polyethylene bags, sealed, cooled and then transported in cooler boxes to the laboratory in Brisbane. Samples were tested individually by ELISA with antibodies specific to CMV, PRSV, SqMV, WMV and ZYMV to determine which virus was present. Percentage virus infection estimates for each individual crop were based on a combination of symptom counts and ELISA results.

Enzyme-linked immunosorbent assay
Leaf samples of cucumber, pumpkin, squash and zucchini were extracted (2g/20ml) in phosphate buffered saline (10mM potassium phosphate, 150mM sodium chloride), pH 7.4, containing 5ml/L Tween 20 and 20g/L polyvinyl pyrrolidone, using a mortar and pestle. The extracts were tested for infection by double antibody sandwich ELISA. Each sample was tested individually in duplicate wells in microtitre plates and appropriate infected and healthy leaf samples were included in paired wells as controls. The substrate used was 0.6mg/mL of *p*-nitrophenyl phosphate in 100ml/L of diethanolamine, pH 9.8. Absorbance values (A_{405}) were measured in a Multiskan plate reader (Labsystems, Finland) and values more than twice those of the healthy control samples were considered positive. The polyclonal antisera to CMV, PRSV, SqMV, WMV and ZYMV used were obtained from BioRad Phyto-Diagnostics, France.

Results and Discussion

A total of 20 farms and 28 cucurbit crops were surveyed in 7 different locations in the 3 main cucurbit growing areas in Qld. Overall, virus infection was detected on 17/20 farms and in 78% of crops. In north Qld, 8/10 farms and 9/12 crops were infected, with individual crop incidences of up to 100%. In the Ayr region of north Qld, PRSV, ZYMV and WMV were all found. PRSV and ZYMV occurred in zucchini crops at Clare and Giru while WMV was also detected in zucchini at Giru. At Mareeba only PRSV was found. In central Qld, 4 of 6 farms surveyed had virus present in 7/10 crops, but PRSV was the only virus found, with individual crop virus incidences of up to 90%. In south Qld (Bundaberg), all of the 6 crops of zucchini on 5 farms were infected with PRSV, and one crop was also infected with WMV. Individual crop overall virus incidences reached 100%.

PRSV was very widespread, being detected in all 7 growing areas on 15/20 farms and in 71% of crops. It was the only virus found in the pumpkin crops sampled in central Qld and in the Mareeba area of north Qld. ZYMV was detected overall in 5/20 farms and 21% of crops. It was only found in north Qld locations where it was present on 5/9 farms and in 50% of crops. WMV was found on 2/20 farms, one each in south and north Qld, and in 7% of crops. SqMV was not detected during the survey but the virus has been recorded previously in pumpkin, squash and honeydew melon in Qld. CMV was found only in a crop of bitter melon *(Momordica*) at Bundaberg in South Qld. This virus is seldom found infecting cucurbits in Qld, unlike the other 4 viruses.

Of the 28 cucurbit crops sampled, 5 had multiple virus infections. Three viruses, PRSV, ZYMV and WMV were detected together in the one zucchini crop in north Qld, suggesting very serious multiple infection. In north Qld, 4 farms with 1 crop sampled on each of them (3 zucchini and 1 squash crop) were co-infected with PRSV and ZYMV. One zucchini crop in south Qld was co-infected with PRSV and WMV.

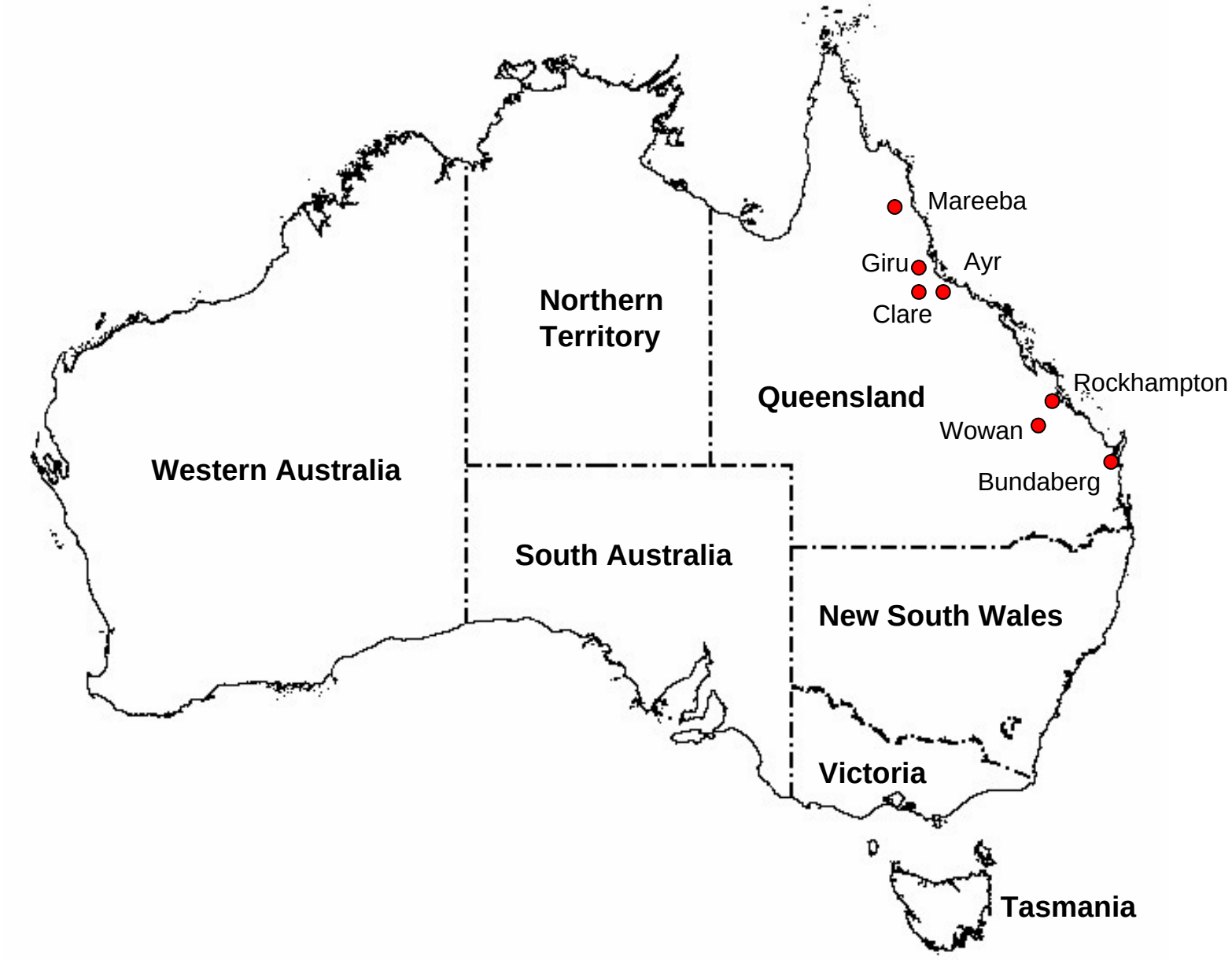
Heavy virus infection was detected in pumpkin, zucchini and squash, with individual crop incidences of up to 100% (zucchini), 90% (pumpkin) and 60% (squash). For pumpkin, 6/10 farms and 11/15 crops were infected, with PRSV being the only virus detected. Three out of the 4 pumpkin crops that were not infected were of susceptible varieities but one was of virus-resistant butternut pumpkin variety Sunset. In zucchini, 8/9 farms and 10/11 crops had virus detected. The 2 crops of squash

surveyed were also both infected. Few crops of squash were available to survey but a high level of virus (60% incidence) was found in one of the 2 sampled. Also, previous work in Qld has shown that squash is often severely affected by PRSV and ZYMV.

Overall virus disease incidence varied from zero to 100% within individual crops, but most crops were virus-infected and the individual infected crops mostly had virus disease incidences above 50%. The incidence of virus-induced mosaic, leaf deformation and stunting symptoms in crops nearing maturity averaged 60% overall while younger crops had lower levels. Both pumpkin and zucchini crops were badly affected by virus in terms of yield reduction and poor fruit quality. Virus disease was particularly serious at Bundaberg during the winter production period. In these Bundaberg crops, virus incidence at harvest was frequently 100% and several crops were not harvested due to poor fruit set and severe fruit distortion. Also, other crops suffered severe yield reduction and high fruit rejection rates from distorted fruit caused by virus infection.

In general, virus disease levels were high in areas where cucurbits were grown on several farms in an area and/or where cucurbit crops were planted in succession with little spatial separation between them. Virus disease incidence was lower were crops where more isolated. High virus levels were favoured by successional plantings near older virus-infected crops or harvested, abandoned crops on the same or neighbouring farms. Frequent insecticide applications, as expected, provided no virus control. Growing virus-resistant varieties should be encouraged as a means of reducing losses due to virus infection. For example, at Rockhampton pumpkin butternut variety Sunset with resistance to PRSV and ZYMV had no virus symptoms compared with an adjacent Japanese pumpkin crop with over 50% infection. The virus-resistant pumpkin variety Dulong which is resistant to the PRSV, WMV and ZYMV, performed well in late season crops in south Qld because of its virus resistance when susceptible ones performed poorly. Virus resistant zucchini varieties have recently become available commercially and their performance should be assessed given the major losses that occur from virus disease in zucchini crops in Qld.

Fig. 1 Locations of cucurbit growing areas in Queensland



SUMMARY – CUCURBIT VIRUS SURVEY 2004, QUEENSLAND

Location	Crop	Virus found[A]			Incidence & comments
		PRSV	ZYMV	WMV	
North Queensland					
Mareeba - Farm 1 (Atherton Tableland)	Pumpkin - Japanese	—	—	—	Nil virus. Maturing crop.
Mareeba - Farm1 (Atherton Tableland)	Pumpkin - Jarrahdale	—	—	—	Nil virus. Maturing crop.
Mareeba - Farm 2	Pumpkin - Jarrahdale	√	—	—	40% incidence. Crop near harvest.
Mareeba - Farm 3	Pumpkin - Jarrahdale	√	—	—	<5% incidence. Crop maturing.
Giru - Farm4	Zucchini cv. Congo	√	√	√	20% incidence. Mature crop
Giru - Farm 4	Squash	√	√	—	50% for each virus Overall incidence 60%
Giru - Farm 5	Zucchini cv. Blackjack	—	√	—	<1% incidence. Mature crop
Clare - Farm 6	Pumpkin - Japanese	√	—	—	c 50% incidence. Advanced fruit set.
Clare - Farm 7	Squash	—	√	—	Trace of incidence. Young crop.
Clare - Farm 7	Pumpkin - Jarrahdale	—	—	—	Nil virus. Mature crop.
Clare - Farm 8	Zucchini cv. Blackjack	√	√	—	45% PRSV, 55% ZYMV infection
Ayr (2003) - Farm 9	Zucchini	√	√	—	100% ZYMV, 50% ZYMV + PRSV infection
Central Queensland					
Wowan - Farm 10	Pumpkin - Jarrahdale	√	—	—	50% incidence. Mature crop.
Wowan – Farm 10	Pumpkin - Japanese	√	—	—	90% incidence. Maturing fruit.

Location	Crop	Virus found[A]			Incidence & comments
		PRSV	ZYMV	WMV	
Wowan – Farm 10	Pumpkin - Japanese	√	—	—	50% incidence. Maturing fruit.
Rockhampton - Farm 11	Pumpkin - Japanese	√	—	—	60% incidence. Near maturity.
Rockhampton - Farm 11	Pumpkin butternut cv. Sunset	—	—	—	
Rockhampton - Farm 12	Pumpkin - Jarrahdale	√	—	—	70% incidence. Mature crop.
Rockhampton - Farm 12	Pumpkin - Japanese	√	—	—	<1% incidence. Next to above Jarrahdale crop.
Rockhampton - Farm 13	Pumpkin - Japanese	√	—	—	60%incidence.
Rockhampton - Farm 14	Pumpkin - Japanese	—	—	—	Nil virus.
Rockhampton - Farm 15	Zucchini	—	—	—	Small in area, isolated crop at harvest.
South Queensland					
Bundaberg – Farm 16	Zucchini cv. Blackjack	√	—	—	100% incidence. Prior to harvest.
Bundaberg - Farm 17	Zucchini cv. Blackjack	√	—	—	70% incidence. Crop at harvest.
Bundaberg - Farm 18	Zucchini cv. Blackjack	√	—	—	1% incidence. Crop at harvest.
Bundaberg - Farm 18	Zucchini	√	—	√	1% incidence. Crop at harvest. 60% incidence PRSV 30% incidence WMV
Bundaberg - Farm 19	Zucchini cv. Blackjack	√	—	—	30% incidence. Crop at harvest.
Bundaberg - Farm 20	Zucchini cv. Blackjack	√	—	—	90% incidence. Crop at harvest.

[A] PRSV = *Papaya ringspot virus*; ZYMV = *Zucchini yellow mosaic virus*; WMV = *Watermelon mosaic virus*

SECTION 3.0

Recommendations

This study revealed a widespread distribution and occurrence of a number of different virus diseases in vegetable cucurbit crops in Northern Australia (WA, NT and Qld). The magnitude of virus infection across cucurbit growing areas was such that it often resulted in serious financial losses to growers. This was not only from virus-induced yield losses but also from obvious quality defects that caused high fruit rejection rates. This situation is a major cause for concern for the vegetable cucurbit industry Australia-wide. Although, much was achieved during the project being reported on here, further action is needed urgently to address the issue which requires developing and communicating an integrated virus disease management strategy to the cucurbit industry.

Recommendations for future research include:

1) Scientific

- Other than melon crops and volunteer vegetable cucurbits, it is vital to establish which alternative virus reservoir hosts (cucurbitaceous and non-cucurbitaceous weeds) are significant sources of virus for spread to cucurbit crops. This is so that the key reservoir hosts can be eliminated by growers thereby reducing virus sources for spread especially in between growing seasons. Removal of such reservoirs is an important component of any integrated virus disease control strategy.

- A number of virus-resistant varieties of zucchini, cucumber and pumpkin have recently come onto the market, but their effectiveness against the four main cucurbit viruses (ZYMV, PRSV, SqMV, WMV) in field situations in Northern Australia still needs to be determined. Field experiments comparing resistance/tolerant varieties with currently grown ones are needed to determine the effectiveness of the virus disease resistance/tolerance, as well as their suitability to growing conditions, their marketability, their yield and commercial potential.

- The magnitude of virus epidemics in crops is dependent on the presence of nearby virus-infected plants which act as the infection source from which the virus is picked up by aphids and spread to the crop. The proximity of cucurbit crops to this virus source and its size are key factors. Numbers of aphids flying from the virus-infected source plants to the crop become diluted greatly the further apart the two are. Also the presence of a non-virus host barrier crop between infection source and susceptible crop provides a ‘cleansing barrier’ on which aphids feed and lose the virus before moving onto the next crop. Prevailing winds also impact on spread which is often greater downwind. There is an urgent need to determine the widths of ‘safe’ planting distances between virus reservoirs of different

potencies and susceptible cucurbit crops, the effectiveness of incorporating non-host barrier crops between susceptible crops and the impact of prevailing wind direction on virus spread. 'Safe' planting distances, non-host barrier crops and planting upwind are potential components of an integrated control strategy that require field validation.

- Large numbers of feeding pumpkin beetles have often been associated with increased virus symptoms and incidence in vegetable cucurbit field crops. However, it is not known if the pumpkin beetle is an additional beetle vector of SqMV. Pumpkin beetles need to be tested for their ability to transmit this virus to establish if they need to be controlled.

- Virus incidence estimates based on visual observations of virus symptoms in field crops often exceed the incidence determined by ELISA tests suggesting the presence of additional viruses not being tested for. Viruses other than the common five (ZYMV, PRSV, SqMV, WMV, CMV) we tested for are recorded infecting cucurbits overseas (eg. *Cucurbit aphid-borne yellows virus*). Identification of which additional viruses are involved is vital before any serious attempt can be made to control them.

- Growers are concerned that SqMV and other viruses may spread between cucurbit plants in infective sap on cutting implements when fruit of zucchini, squash and cucumber are cut off at harvest. The extent of spread of virus to healthy plants via cutting implements needs to be determined, as does the effectiveness of different disinfectants for cutting knives in preventing spread. Suitable control measures can then be devised as needed, and incorporated within an integrated control approach.

- Inadvertent planting of SqMV-infected seed stocks by growers results in potent sources of infection scattered through the crop from which virus epidemics commence early. The restriction of SqMV occurence to northern WA in our survey suggests that the seeds being used in this region are probably contaminated (SqMV is a seed-borne virus). An effective, routine seed test for SqMV needs to be developed. This would then be used to establish whether commercial seed lots are virus-contaminated before they are sown. Contaminated seed lots could then be avoided.

2) Industry

This study revealed widespread distribution and occurrence of at least 5 different virus diseases in vegetable cucurbit crops in Northern Australia (WA, NT and Qld). The magnitude of the losses in yield and quality, and the resulting financial losses being experienced by growers are cause for concern to the industry. The study also highlighted the lack of effective virus management strategies available for use by cucurbit growers to help manage these damaging virus diseases.

There is an urgent need for research to develop and validate multi-faceted integrated disease management strategies and information packages for each of the main vegetable cucurbit crops (squash, zucchini, cucumber and pumpkin). Such strategies would need to take into account the distinct virus diseases prevalent in different regional locations. A major emphasis on raising awareness of virus disease issues and management strategies then needs to be undertaken. This would inform the Australian vegetable cucurbit growers of the extent of the problem, why it is occurring and the actions they need to take to address it such that the current financial losses from low yields and fruit rejections due to quality defects can be avoided.