Integrated Viral Disease Management in Vegetable Crops

Denis Persley
The Department of Agriculture,
Fisheries and Forestry, QLD

Project Number: VG07128

VG07128

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

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

ISBN 0 7341 2868 1

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

HAL

Horticulture Australia

FINAL REPORT

INTEGRATED VIRAL DISEASE MANAGEMENT IN VEGETABLE CROPS

VG07128 (Completed May 30 2011)

Denis Persley *et al.*

Agri Science Queensland-Department of Employment, Economic Development and Innovation

Horticulture Australia Ltd Project Number VG07128

Project Leader: Denis Persley, Principal Plant Pathologist, Department of Employment, Economic Development and Innovation (DEEDI)
Horticulture and Forestry Sciences
Ecosciences Precinct
Dutton Park QLD 4102
GPO Box 46 Brisbane 4001 Australia
Ph: 3255 4388
E mail: denis.persley@deedi.qld.gov.au

Project team members:
Dr Cherie Gambley, Senior Plant Pathologist, DEEDI Ecosciences Precinct Dutton Park QLD 4102
Dr John Thomas, Principal Research Fellow, University of Queensland, Queensland Alliance for Agriculture and Food Innovation (QAAFI), Ecosciences Precinct, Boggo Road Dutton Park QLD 4102.
Brenda Coutts, Senior Virologist, Department of Agriculture and Food Western Australia, Baron Hay Court, South Perth WA
Dr Roger Jones, Principal Plant Virologist, Department of Agriculture and Food, South Perth WA
Dr Calum Wilson, Tasmanian Institute of Agricultural Research, Hobart Tasmania 7008.
Dr Brendan Rodoni, Senior Plant Virologist, Department of Primary Industries, Knoxfield Victoria
Dr Len Tesoriero, Industry Leader, Greenhouse and Ornamental Crops, Elizabeth Macarthur Agricultural Institute, Menangle NSW 2568
Dr Leanne Forsyth, Plant Pathologist, Elizabeth Macarthur Agricultural Institute NSW. (now DEEDI QLD).
Tony Burfield, Extension Horticulturist, South Australian Research and Development Institute, Adelaide SA.
Iain Kay, Principal Entomologist, DEEDI Bundaberg QLD.

Purpose: This report provides information on the identification, biology and management of virus diseases in vegetable crops in Australia. The report describes measures taken to increase adoption of an integrated approach to virus management in the industry.

Date of report: May 30 2011

Funding sources: This project has been funded by HAL using the vegetable industry levy and matched funds from the Australian Government.

Agri Science Queensland (DEEDI)
New South Wales Department of Industry and Investment
Department of Primary Industries, Victoria
Tasmanian Institute of Agricultural Research
Department of Agriculture and Food Western Australia

Contents

Media summary

Surveys as part of the project have found that virus diseases are an important cause of loss to the vegetable industry. Crops which are often severely affected include vegetable cucurbits (papaya ringspot, watermelon mosaic and zucchini yellow mosaic viruses); capsicums (Tomato spotted wilt virus (TSWV)) and lettuce (TSWV; lettuce big vein disease).

Management of viral diseases requires an integration of several methods aimed at preventing or delaying infection of crops. Plants cannot be cured once infected. Best practice guidelines have been prepared by industry experts, in conjunction with research on disease management, as part of an integrated disease management project. Information on best practice is been provided to growers and consultants through reference material, technical papers, regional information sessions, field days and personal networks.

Technical summary

The aim of project VG07128 was to enhance the capacity of the vegetable industry to implement integrated viral disease management and reduce the economic impact of these diseases to the industry.

Strong collaborative work by plant virologists in all States identified virus diseases as key issues in vegetable production in both field and protected cropping systems. The major economic losses were found in capsicum (Tomato spotted wilt virus, Cucumber mosaic virus, Tobamovirus), all vegetable cucurbits (potyviruses and Beet pseudoyellows virus in cucumber), lettuce (big vein disease), beans (Bean common mosaic virus at Kununurra) and brassicas (Turnip mosaic virus in some areas).

Tomato crops also had significant virus problems including Tomato yellow leaf curl virus in south Queensland and torrado virus in protected cropping in southern Australia. These viruses can also infect other Solanaceous species and need to be addressed as part of area wide management plans.

Surveys over three years of processing pea, bean and brassica crops in Tasmania found relatively low levels of virus infection, suggesting that virus diseases currently have a minor impact on processing crops in the State. The survey, however, highlighted potential key viral pathogens that may require further monitoring and assist in maintaining high health and quality of processed vegetable crops in Tasmania.

Several virus diseases were identified for the first time in Australia including Tomato torrado virus and Ranunculus white mottle virus causing capsicum yellow vein disease. In addition to benchmarking surveys, experimental work provided new information on virus spread by contact, virus tolerant zucchini varieties as a means of enhancing disease management and reducing pesticide use and the cause and impact of lettuce big vein disease.

An increased awareness of virus diseases and there management has been provided through industry seminars, personal contact at all levels of the vegetable industry and the publication of six reference notes which form the basis of a guide to the integrated management of virus diseases in vegetables. The industry seminars, some of which have involved training in virus identification and management, have been held in all mainland states and have targeted all areas where virus diseases are a production problem. The published material has been linked to the knowledge management system through the vegetable industry development program. The progress made needs to be continued to further promote the integration of viral disease management in crop and area wide crop protection plans.

Introduction

The Australian vegetable industry is valued at approximately 3.4 billion dollars. The industries within the vegetable sector can be grouped as follows:

- Leafy and Asian vegetables
- Brassica vegetables
- Cucurbit vegetables including melons
- Tomato and capsicum
- Sweet corn, asparagus, beans and peas
- Fresh and processing potatoes
- Carrots and onions
- Protected cropping

Vegetable production is important in all States with each having a major role in the production of one or more crops. Queensland, for example, is the major supplier of capsicum, sweetpotato, fresh tomatoes and several cucurbits, e.g. melons, zucchini and pumpkin. Tasmania is a major producer of beans, peas and potatoes for processing. Victoria is the leading producer of brassica vegetables, lettuce and celery while New South Wales is the leading producer of sweet corn for processing and fresh market trade. Protected cropping and hydroponic production is an increasing segment of the industry, particularly for tomatoes, cucumbers, lettuce and capsicums. Covered production of cucumbers currently accounts for 46% of total production.

Crop protection has always been a significant component of profitable vegetable production and the impact of virus diseases has been an important issue over many years in several sectors of the industry. Virus diseases are generally difficult to manage as many viruses have active insect vectors facilitating rapid spread, plants cannot be cured once infected hence management needs to be aimed at preventing or reducing infection and resistant varieties are often not available to protect against important viral pathogens.

Among the first virus diseases to affect Australian vegetable crops were those caused by *Tomato spotted wilt virus* (TSWV) which was first described from tomato in Victoria (Brittlebank1919). The virus caused serious losses in all Australian States during the 1920s and 1930s, particularly in tomato crops (Samual 1930). Severe epidemics also occurred in potato crops in NSW and Victoria in 1945-46 and 1946-47 with disease levels of up to 60% and the rejection of 31% of crops examined for seed certification in NSW (Norris 1951).

TSWV remains one of the most widespread and damaging viruses in Australia. The incidence of TSWV in vegetable crops began to increase in the 1990s and continued to do so into the 21st century (Latham and Jones 1996; Wilson 1998; Persley et al. 2006) A significant factor in resurgence of the pathogen was the entry into Australia of the western flowers thrips (*Frankliniella occidentalis*) in 1993, a proven efficient vector of TSWV. The crops most severely affected by TSWV include tomato, capsicum, lettuce and potato. Several severe epidemics occurred in vegetable crops on the North Adelaide Plain in 2000 with estimated losses of $70M (Anon 2000) and in the Perth metropolitan area in 2001/2 where crop failure of capsicum, lettuce and tomato was common due to TSWV(Coutts and Jones 2002).

Several other important vegetable crops had significant virus disease problems in the years leading up to 2000.

Carrots were severely affected by motley dwarf disease in Victoria and other States in the 1940s and 50s, particularly varieties derived from Chantenay stock. Crops frequently had a high percentage of plants that were stunted with mottled, distorted foliage and poor root development (Stubbs 1948; 1952). The disease was later shown to be due to infection by two viruses, the luteovirus *Carrot red leaf virus* and the umbravirus *Carrot mottle virus*, both of which were transmitted by the aphid *Cavariella aegopodii* in a persistent manner (Murant 1974, Waterhouse and Murant 1982). By the mid 1960s the disease had been well controlled through the selection of virus tolerant or aphid repellent varieties, a change in sowing dates to avoid seasonal flushes of the carrot aphid, the widespread use of insecticides on carrots and the effectiveness of an introduced parasite on the carrot aphid (Buchen-Osmond et al. 1988).

Aphid –transmitted potyviruses have caused sporadic epidemics in celery over several decades. Alberts et al (1989) reported a severe outbreak of *Celery mosaic virus* around Adelaide during 1986/87 where up to 70 % of plants in crops were affected, resulting in yield losses and downgrading of produce. *Celery mosaic virus*, sometimes in combination with *Cucumber mosaic virus*, caused extensive losses on the Mornington peninsula in Victoria in the late 1990s. This prompted an examination of potyviruses infecting Apiaceae in Australia (Moran et al.2002) and three distinct viruses were identified by gene sequencing: *Celery mosaic virus* from celery, *Carrot virus Y* from carrot and *Apium virus Y* infecting parsley, sea celery and several weedy species of Apiaceae. *Carrot virus Y* was subsequently found in six States and, as with *Celery mosaic virus*, was more likely to be damaging when carrot or celery crops where grown throughout the year (Latham et al. 2004, Latham and Jones 2004).

Lettuce crops have been severely damaged by virus diseases for many decades in all States. Although TSWV and *Cucumber mosaic virus* caused sporadic losses, epidemics of *Lettuce mosaic virus* (LMV) and *Lettuce necrotic yellows virus* (LNYV) were the most frequent cause of crop failures (Stubbs and Grogan 1963). The symptoms of TSWV, LMV and LNYV are very similar in lettuce and detailed work by Stubbs and Grogan (1963) demonstrated that LNYV was a previously undescribed virus transmitted persistently by the sowthistle aphid (*Hyperomyzus lactucae*) and having sowthistle (*Sonchus oleraceus)* as the major alternative host of both the virus and vector. Stubbs et al. (1963) also found that destruction of sowthistle in and around lettuce crops was an effective means of managing the disease.

Bean summer death disease caused by the geminivirus *Tobacco yellow dwarf virus* caused major losses in susceptible bean cultivars, particularly in areas west of the Great Divide after periods of hot dry weather favoured the migration of the leafhopper vector *Orosius argentatus* from alternative hosts into bean crops (Ballantyne 1968; Thomas and Bowyer 1984).

Vegetable cucurbit and melon crops in northern Australia have frequently developed high levels of virus disease since at least the 1960s. *Watermelon mosaic virus* (WMV) was the dominant virus until about 1970 (Greber1969) when *Papaya ringspot virus*-type W was detected and caused considerable damage to watermelon and pumpkin crops (Greber

1978). The economic impact of viruses on cucurbit production increased further when *Zucchini yellow mosaic virus* was found in 1987 (Greber et al 1988).

Despite periodic outbreaks of TSWV and *Tomato mosaic virus*, the most prevalent and damaging virus in tomato and capsicum crops in Queensland for some 40 years was *Potato virus Y* (Sturgess 1956; Thomas et al 1989). The virus occurred in all production areas and surveys frequently revealed incidences of 50% to 100% in both tomato and capsicum. Several strains virulent towards resistance sources in capsicum were also identified (Thomas et al. 1989).

The information above is provided as a background to work undertaken in VGO 7128 and will allow a comparison between viruses currently having an economic impact and those of concern some years ago.

The overall aim of VGO 7128 –Integrated management of viral diseases in vegetable crops was to enhance the capacity of the vegetable industry to implement integrated viral disease management and reduce the impact of virus diseases in a range of vegetable crops.

Materials and Methods

Disease surveys: Comprehensive surveys were undertaken by random sampling of 300 plants in a crop or adjacent crops. Individual symptomatic leaves or fruit were also sampled as appropriate. Smaller sample sizes and individual specimens submitted for disease diagnosis were also included in the surveys to broaden the crops and areas sampled. Samples were stored in plastic bags, labelled and kept cool before transport to a laboratory. Information was collected with a survey form on location (GPS coordinates or address), contact details, stage of growth, cultivar, crop area, disease symptoms and estimated incidence, vector type and numbers present, abundance and dominant weed species in the crop area.

Virus identification was done using ELISA serological tests, polymerase chain reaction (PCR) using virus group or specific primers, gene sequencing, electron microscopy and biological assays to determine host range or strain type.

Crops in the following vegetable production areas were surveyed at least once during the project:

Queensland
Bowen/ Burdekin region- capsicum, tomatoes
Rockhampton-capsicum, sweetpotato, vegetable cucurbits
Bundaberg-capsicum, tomato, eggplant, sweetpotato
Lockyer valley-capsicum, lettuce, brassicas,
Granite belt-lettuce, capsicum, brassicas

New South Wales
Sydney basin-lettuce, vegetable cucurbits (cucumbers, zucchini)
Far North Coast-cucumbers (protected cropping), capsicum, herbs
Bathurst-brassicas, lettuce

Victoria
Mildura/ Swan Hill-vegetable cucurbits (zucchini, pumpkin), capsicum, eggplant, tomato
East Gippsland-lettuce
Metropolitan-lettuce, brassicas, tomato

South Australia
North Adelaide Plain (Virginia region)- capsicum, lettuce, tomato

Western Australia
Kununurra (tropical north)-vegetable cucurbits, beans
Carnarvon (sub-tropical mid north)-vegetable cucurbits, capsicum , tomato, eggplant
Temperate south west (Manjimup, Gingin, Carabooda)-brassicas, capsicum, chilli, carrots, celery, lettuce, tomato, spinach, beet.

Tasmania
Northern cropping region- processing pea and bean crops, brassicas

Results

A summary of results from surveys for virus diseases over the period 2008 to 2011 is provided under crop headings.

Brassicas (cabbage, cauliflower, broccoli)
Turnip mosaic virus (TuMV), an aphid transmitted potyvirus, was widespread in brassica crops in the Sydney basin and at Bathurst in the winter of 2008/09, with multiple crops having a virus incidence of 100%. The virus was particularly damaging in cabbage as the virus causes necrotic ringspots on the outer leaves during cool weather, requiring removal of the affected leaves before marketing. This results in a downgrading of size and quality. TuMV was frequently found in Brassicaceae weeds adjacent to affected crops, for example wild turnip (*Brassica tournefortii*), turnip weed (*Rapistrum rugosum*) and wild radish (*Raphanus raphanistrum*). These weed species provide a source of inoculum and virus reservoirs for virus survival over summer when few field brassicas are grown in these areas.

TuMV was not found in a broccoli crop at Bathurst, despite adjacent cabbage crops having high levels of viral infection, suggesting the cultivar may have resistance to the TuMV strain present.

In Tasmania brassica crops (broccoli, cauliflower) were tested for potyvirus, luteovirus, *Cucumber mosaic virus* and *Cauliflower mosaic virus*. The 24 broccoli crops surveyed over three years had less than 2% infection by *Beet western yellows virus*. A single infection of *Cauliflower mosaic virus* was detected in one of five cauliflower crops surveyed in 2008.

Crops of cauliflower and broccoli at Manjimup WA were abandoned due to small and distorted heads following an epidemic of *Beet western yellows virus* (BWYV). The epidemic appeared to be due to large aphid flights in October / November which carried the virus from infected wild radish into cauliflower crops. Transplants had not been treated with insecticide thus allowing colonisation of young plants by aphids.

A low incidence of *Cauliflower mosaic virus* was detected in one crop of cauliflower at Kununurra.

Cucurbits (pumpkins, squash, zucchini, cucumbers)

Surveys and related project work by team members have shown that virus diseases are an important cause of crop failure and reduction in yield and quality of vegetable cucurbits.

In WA, the aphid transmitted potyvirus *Zucchini yellow mosaic virus* caused major losses in cucumber, pumpkin, squash, watermelon and zucchini crops at both Kununurra and Carnarvon in 2009 and 2010. At both locations, many crops were abandoned due to virus infection towards the end of the winter growing season with major economic losses to growers.

In the Swan Hill area of north- west Victoria, zucchini crops were surveyed over three years for five viruses (*Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV). Virus incidence in zucchini crops ranged from <1% to 100% infection. *Watermelon mosaic virus* was the only virus found in ELISA tests. At the survey times in February of 2008 and 2009 virus incidence greater than 50% was frequently found in crops at harvest stage. Although WMV does not cause the severe fruit symptoms seen with PRSV and ZYMV, it does cause skin mottling and slight distortion resulting in downgrading of produce. The yields of crops infected early are also reduced. In 2010 virus incidence was lower and growers had planted larger areas of virus tolerant cultivars as seed of the widely used cultivar Congo was in short supply.

Over a two year period seven farms producing zucchini in the Sydney basin were surveyed for PRSV, ZYMV and WMV. All three potyviruses were identified. The incidence of WMV and ZYMV peaked in February-March and decreased into autuum while PRSV levels were generally lower in late summer and peaked in May.

Virus incidence varied from <2% to 100% infection for each virus. Some reasons for this wide variation in disease levels include variations in aphid populations and activity, the amount of inoculum available e.g. older infected crops adjacent to young crops, changes in zucchini cultivars during the season and harvesting practices. A substantial increase in virus incidence from harvest onwards may be the result of both aphid transmission and virus spread on cutting implements.

Surveys and testing of submitted samples over ten years in Queensland confirmed Papaya ringspot virus type W as the most widespread and dominant virus infecting cucurbits. This virus frequently causes crop failure or considerable economic losses in zucchini, watermelon and, to a lesser extent, pumpkin crops in south Queensland. Both PRSV-W and Zucchini yellow mosaic virus occur in the Bowen / Burdekin region of north Queensland. Melon, zucchini, squash and pumpkin crops are regularly severely affected. In 2010 all samples from a zucchini crop near Ayr were positive by ELISA for PRSV-W and 40% of samples were also infected with ZYMV.

Cucumber crops grown under cover frequently develop yellow blotchy raised areas between the veins on older leaves with downward curling of leaf margins. Symptoms gradually progress to the younger leaves and plants lack vigour. Although these

symptoms are similar to those caused by nutrient deficiencies or poor growing conditions, the whitefly transmitted crinivirus *Beet pseudoyellows virus* (BPYV) has been detected by PCR tests in many plants with these symptoms. BPYV was first reported from Australia in several weed species in Tasmania in 1981 and the current work has found that the virus is widespread and frequently common in cucumber crops on the North Adelaide Plain, in the Sydney basin and at Coffs harbour on the far north coast of NSW. The effects on production have not been quantified but crops with a high incidence of early infection lack vigour and have reduced fruit set and poor quality fruit.

Solanaceous crops (capsicum, eggplant, tomato).

The viruses causing economic losses to capsicum crops in Australia are the thrips transmitted tospoviruses *Tomato spotted wilt virus* (TSWV) and *Capsicum chlorosis virus* (CaCV) and the aphid transmitted *Cucumber mosaic virus* (CMV).

At Carnarvon (WA) in 2010, CMV and TSWV were both detected in capsicum, chilli and eggplant crops at incidences of up to 100% and 80% for CMV and TSWV, respectively. Many crops were abandoned because of low yields and unmarketable fruit. The major outbreaks of TSWV were linked to high populations of western flower thrips.

In the south west of WA, TSWV was detected in capsicum and chilli crops with disease incidences of up to 50%. Most crops are TSWV resistant hybrids and one greenhouse crop of a resistant hybrid developed 50% infection with TSWV, indicating the development of a resistance breaking strain of TSWV.

In the Virginia area of the North Adelaide Plain, TSWV remains the major cause of loss from disease. A resistance breaking strain of TSWV is dominant in crops and disease incidence frequently exceeds 50% as crops mature. All available hybrids are susceptible as the virus strain is virulent towards the *Tsw* gene, the only source of TSWV resistance available in commercial breeding programs.

At Bundaberg (Qld), CaCV has been the dominant virus in capsicum crops for over five years with very few detections of either TSWV or the potyvirus Potato virus Y during multiple surveys over that period. The incidence of CaCV in capsicum crops is generally less than 10% but incidences of over 50% have been found where crops are close to infestations of *Ageratum conyzoides*, an important alternative host for the virus. A level of 5% infection by CaCV results in approximately a 5% loss of production as fruit set is reduced and fruit that do develop are unmarketable.

Both CaCV and TSWV occur in capsicum crops in the Burdekin/ Bowen area, causing sporadic losses each year. A low incidence of CaCV has been detected in capsicum crops at Kununurra on several occasions.

Since 2009 several growers in the Virginia area have suffered considerable losses from a disease causing skin distorting and mottling of fruit, making the fruit unmarketable or reducing quality. Laboratory tests confirmed *Tomato mosaic virus* (ToMV) as the cause of the disease and surveys determined the numbers of farms involved and advice was provided to growers to help contain the outbreak. The source of the virus has not been determined but ToMV is easily spread on contaminated seed, implements and clothing.

A virus-like disease causing bright yellow vein banding and chlorosis has been seen in many capsicum crops in greenhouses near Virginia since at least 2000. Molecular and biological tests have shown that this disease is caused by the Ophiovirus *Ranunculus white mottle virus*. This virus infects plants through the roots, most likely via the soil borne fungus *Olpidium*. This was the first record of the virus in Australia and the first on capsicum worldwide.The disease, named capsicum yellow vein disease, although widespread on the North Adelaide Plain in capsicum crops does not appear to cause significant yield loss or reduction in fruit quality. Disease incidence can be up to 30% with symptoms easily seen in young plants and fading as plants mature. The disease has not been seen elsewhere in Australia.

The begomovirus Tomato yellow leaf curl was first detected in Australia in south east Queensland in 2006 where it is now a major limiting factor to tomato production, particularly in the Bundaberg area. Disease incidence is frequently 80% to 100% by harvest time in both gourmet and cherry tomato cultivars. Crop losses are at least several million dollars per season through yield reduction and wasteage due to undersize fruit, particularly in cherry tomatoes.

Management of the whitefly vector, *Bemisia tabaci* B biotype provides some control but the introduction of adapted varieties with TYLCV resistance is critical. The virus was found in northern NSW in 2010 and at Mareeba in north Queensland in 2011, placing the Bowen tomato production area at greater risk.

The cause of a disease present in greenhouse tomato crops on the North Adelaide plain since at least 2005 was identified as *Tomato torrado virus*, a whitefly transmitted virus belonging to a new genus Torradovirus. This virus, transmitted by the greenhouse whitefly *Trialeurodes vaporariorum*, causes severe necrosis and chlorosis on highly susceptible cultivars. The virus was found in greenhouse tomato crops near Geelong (Vic) in 2010. The virus has caused sporadic serious crop losses but can be managed with resistant cultivars and whitefly management.

TSWV occurs sporadically in tomato crops in all States, although the losses seem less than in capsicum. In south west WA TSWV occurred in tomato crops with levels of up to 50%. Most cultivars grown are TSWV resistant, following major crop failures from TSWV in recent years. In 2010 a resistance breaking strain was found in the cultivar Swanson which has the *Sw-5* resistance gene. This is the first record of a TSWV field isolate virulent on the *Sw-5* gene in Australia.

Potato spindle tuber viroid was identified by PCR and sequence analysis from greenhouse grown tomato plants on the Sunshine coast, Queensland in April 2011.

Legumes (peas, beans)

Pea and bean crops in Tasmania were surveyed over three years for potyviruses, luteoviruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Subterranean clover stunt virus* (SCSV) and TSWV.

Low and sporadic incidence of each virus or virus group were detected in the pea crops sampled. Only Luteovirus and SCSV were found at a low level in bean crops. In the pea crops Luteoviruses were the most prevalent virus group detected.

Samples testing positive for Potyvirus and Luteovirus infection were retested using antiserum specific to known virus species. These tests revealed all potyvirus detections (from pea crops in the first year of testing) were due to infection with *Pea seed-borne mosaic virus* (PSbMV). These samples were all sourced from a single imported seed line. Subsequent testing over the next two seasons failed to show evidence of PSbMV which may have been eliminated with destruction of the seed source. Serological retesting of Luteovirus samples revealed 32% of samples tested positive for *Beet western yellows virus* (BWYV), whilst *Bean leafroll virus* and *Soybean dwarf virus* were not detected.

Molecular analyses of luteovirus isolates collected from these crops revealed most infections were due to *Turnip yellows virus* (a variant of BWYV) with a few isolates. from pea belonging to a putative novel luteovirus species with closest homology to *Curcurbit aphid-borne yellows virus.*

In Kununurra, crops of borlotti beans were destroyed due to epidemics of *Bean common mosaic virus* in 2009 and 2010, causing significant losses in this industry valued at approximately $5M per year.

Carrots, celery

The incidence of virus in these crops was low during the survey period. Potyvirus e.g. Carrot virus Y, CMV and AMV were not detected in carrot crops surveyed in Tasmania in 2008/09 season. A trace (<1%) amount of *Carrot virus Y* was found in one carrot crop in the south –west of WA. Celery mosaic virus, although present in Queensland (Granite Belt) and Victoria (Mornington peninsula), is at a lower incidence than in the late 1990s when the virus was having considerable economic impact on the industry, particularly in Victoria.

A celery crop located in the Lockyer valley was surveyed in late winter 2008 and a crop from the Stanthorpe region was surveyed in autumn 2009. From each crop 300 samples were collected from randomly selected plants and then divided into 30 bulk samples, each containing 10 individual samples. The 30 bulk samples were then indexed for CeMV by DAS-ELISA using CeMV specific antisera.

No obvious virus was observed in the Lockyer valley crop and CeMV was not detected in any of the bulk samples. However, the Stanthorpe grown crop had approximately 2% of plants with symptoms of CeMV and the virus was detected in eight of the 30 bulk samples. The virus was also detected in three individual samples collected from symptomatic plants. These three samples were further tested by RT-PCR using potyvirus degenerate primers and the expected DNA fragment was obtained from all three samples. Two of these amplicons were sequenced and confirmed 100% identical to previously described sequences of CeMV from Australia.

Lettuce

Lettuce surveys were concentrated in Victoria (East Gippsland; Werribee; Morington peninsula) and Queensland (Lockyer valley; Stanthorpe), although crops were also surveyed and isolates collected from South Australia, WA and NSW .Lettuce big vein disease was found in all growing areas in both field and hydroponic production. The incidence varied from trace infection to total infection of crops. Symptoms also varied from mild to very severe and prominent clearing of veins, making plants unmarketable. Both *Lettuce big vein virus* and *Mirafori lettuce virus* were found in all areas and their relative importance in causing the disease requires further investigation, although data suggests that MiLV is the causal agent of the disease.

Other viruses found in surveys were *Lettuce necrotic yellows virus*, *Turnip mosaic virus*, TSWV and *Cucumber mosaic virus*, all of which have the capacity to cause sporadic disease outbreaks in all production areas.

Other survey results

The tospovirus *Impatiens necrotic spot virus* was detected by ELISA and sequence comparisons in begonia, spathiphyllium, lisianthus, chrysanthemum and basil in a nursery near Gosford. Eradication measures were undertaken as this was the first detection of the virus in Australia. The establishment of the virus in Australia would be a threat to the vegetable industry as this virus can infect several vegetable crops including lettuce, spinach and potato. Surveys of vegetable crops near the infected site did not detect INSV.

Natural infection of fleabane *(Conyza* species), dock *(Rumex obtusifolius*) and sowthistle (*Sonchus oleraceus*) by Papaya ringspot virus was confirmed by ELISA and sequence data from samples collected in the Sydney basin. These are the first records of natural infection of species outside of Cucurbitaceae and Caricacae.

ZYMV and WMV were detected in artichoke in the same surveys.

The potyviruses Apium virus Y and Clover yellow vein virus were found in coriander having chlorotic blotching and interveinal chlorosis.

A strain of TSWV able to infect capsicum hybrids with the *Tsw* resistance gene was found at several widely separated locations in coastal Queensland in the introduced weed Jamacian snakeweed (*Stachytarpheta jamaicensis*) and appears to have evolved in the absence of selection pressure from resistance genes.

A resistance breaking strain of TSWV was found in Sonchus oleraceus growing in a fallow greenhouse area in July 2009 indicating that the RB strain dominant in capsicum in Virginia can survive in weed species thus providing a means of survival between crops.

Research

Summary of research activities

- Zucchini plots separated by a 15m barrier of bare ground from a source of *Papaya ringspot virus* delayed infection during the early growth stages. A barrier of shade cloth had only a marginal effect on delaying virus spread.

- *Zucchini yellow mosaic virus* (ZYMV) was easily spread among zucchini plants by leaf contact and on contaminated cutting implements. Mechanical spread of ZYMV and, most likely, related viruses is considered to be more important than previously recognised.

- The crinivirus *Beet pseudoyellows virus* was consistently associated with interveinal chlorosis, brittle leaves and stunting in greenhouse grown cucumber crops in NSW and South Australia. Although previously reported from dandelion (*Taraxacum officinale)* in Tasmania this work indicates that this whitefly transmitted crinivirus is widespread in greenhouse cucumber crops in southern Australia. The virus was also found in the weeds *Chenopodium murale*, *Lactuca serriola* and *Capsella bursa-pastoris*.

- *Tomato mosaic virus* was identified by ELISA and sequence comparisons as the cause of fruit distortion and mottling in capsicum crops around Virginia SA. Twenty hybrids were screened for resistance by sap inoculation with most producing a hypersensitive resistant response. Extension information was written and grower meetings were held to assist in limiting spread of the disease.

- Zucchini cultivars with tolerance to *Papaya ringspot virus* were identified in a field trial in south Queensland. Cultivars with high tolerance allow economic yields under high virus disease pressure and are a valuable tool in the management of aphid transmitted potyviruses in zucchini.

- Isolates of *Lettuce big vein virus* and *Mirafori lettuce virus* were collected and characterised from field and hydroponic crops with lettuce big vein disease throughout eastern Australia. Soil transmission studies with Olpidium were undertaken. The work indicated that further work is required on the role of the two viruses in the etiology of LBVD.

- A disease causing conspicuous yellow vein banding symptoms in capsicum on the North Adelaide Plain was graft transmitted to capsicum and also transmitted to young capsicum plants grown in soil from under field infected plants. PCR tests and sequence analysis identified the Ophiovirus *Ranunculus white mottle virus* as the cause of these symptoms. This is the first record of the virus in Australia and on capsicum worldwide.

- A desk top study identified the factors associated with climate change and variation in the incidence and severity of plant viruses in vegetable crops. An advanced draft of a manuscript was produced.

Patterns of spread of *Papaya ringspot virus* in zucchini.

Brenda Coutts and Roger Jones. Department of Agriculture and Food, Western Australia

Introduction

The principal viruses infecting cucurbit crops in Australia are *Papaya ringspot virus* –type W (PRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV), all from the Potyviridae family of plant viruses (Persley and Horlock 2003; Coutts and Jones 2005; Coutts *et al.* 2011). Plants infected with these viruses have distorted leaves with mosaic and fruit are small, lumpy and distorted. All of these viruses cause severe foliar and fruit disease in cucurbit crops resulting in substantial yield and quality losses (Greber *et al*. 1988; Herrington 1987; Coutts and Jones 2005; Coutts *et al*. 2011). The three viruses are non-persistently aphid transmitted with *Myzus persicae* (green peach aphid) and *Aphis gossypii* (melon aphid) being the main vectors. They also infect volunteer cucurbits and wild cucurbits including Afghan melon (*Citrullus lanatus*) and wild gerkin (*Cucumis angurin*) (Persley and Horlock 2003; Coutts and Jones 2005).

Management of these viruses includes destroying old cucurbit crops, weeds and volunteer cucurbits that act as virus and vector reservoirs, avoid overlapping plantings, use of reflective mulch to deter aphid landing and use of resistant varieties. Insecticide application is ineffective at decreasing their spread within cucurbit crops (McLean *et al.* 1982; Coutts and Jones 2005; Persley *et al*. 2010; Coutts *et al*. 2011). Cultural control measures such as planting upwind of virus sources and use of barriers have been shown to delay virus spread to crops (eg Coutts *et al*. 2004; Jones *et al*. 2005; Coutts *et al.* 2011). To help further develop an integrated disease management strategy for cucurbit potyviruses studies on spatial patterns of virus spread are required.

This study describes two field trials in which bare earth fallow or a non-host barrier separated PRSV-infected cucurbit source plants from cucurbit plots planted at different distances from them. PRSV is being used here but it functions as a model system for ZYMV and WMV.

Materials and Methods

The Qld-1isolate of PRSV used was supplied by Denis Persley and was from Queensland. The PRSV culture was maintained in zucchini cv. Blackjack plants and was used to produce infector plants of zucchini for transplanting to the field and for positive controls in ELISA. Polyclonal antiserum specific to PRSV from Loewe Biochemica

(Germany) was used. Virus culture plants, healthy transplants and infector plants of zucchini cv. Blackjack were grown from seed in potting mix in air-conditioned, insect proofed glasshouses. To produce infector plants, plants were sap inoculated with isolate Qld-1, at the 2 true-leaf stage. Leaf samples were tested by ELISA as described by Coutts and Jones (2005).

Field trials were done at Department of Agriculture and Food irrigated field plots at South Perth. For each trial a bay 10.5 x 50m was used. For trials 1 and 2, 10 PRSV infector plants were transplanted on 8 December 2009 and 29 January 2010 respectively into a 1 m wide x 2 m long plot running across the middle of the bay. At the same time, 10 healthy 4-week old zucchini plants were transplanted into each of 12 other plots. Each of these plots was 1 m wide x 2 m long and consisted of two raised beds 1m apart, with 0.5m plant spacing within beds. Six (trial 1) or four (trial 2) of these plots were arranged around the PRSV 'source' plot such that they all started 1m away from it and radiated outwards, three (trial 1) or two (trial 2) each to the east and west, with a 1m space between each plot. Another six (trial 1) or eight (trial 2) identical plots started 15m away from the 'source' plot and radiated outwards, three (trial 1) or four (trial 2) each to the east and west, with a 2 m (trial 1) or 3 m (trial 2) space between each of them. In trial 2, a 1.2 m high shade-cloth barrier was erected around two plots each to the east and west. The bays were kept weed free to maintain a bare earth fallow around plots.

In trial 1, at 35 days after planting, and for trial 2, at 14, 17, 20, 24, 28 and 32 days after planting a young leaf sample was collected from each plant and tested individually by ELISA for PRSV. Symptoms were recorded at the same time. In trial 2, aphids were collected from zucchini plants on one occasion for identification.

Fig 1. a) Trial 1 with three plots in the foreground 15 m to the west of the the central source plot which had six plots starting 1m from it (three on ether side) and three plots 15 m to its east. b) Trial 2 (at an earlier growth stage than trial 1) with four plots 15 m to the east of the source plot, two with shade-cloth barrier (right) and two without (left) in the foreground, the central source plot with four plots on either side (i.e. to its north and south) starting 1m away from it and four plots 15 m to the east of the source plot, two with shade-cloth barrier (right) and two without (left).

Results

Trial 1

All zucchini plants were infected with PRSV at 35 days after planting. All plants developed symptoms of severe leaf distortion and mottle.

Trial 2

By 17 days after planting, all plants in all four plots sited 1m from the virus source were PRSV-infected, symptoms of mild mottle being visible at this time (Fig. 2). There was much less spread to plots 15m away from the source but there was little difference between those to the east and those to the west. Plots with the shade-cloth barrier had slightly lower virus incidence at 17-28 days after sowing than those without the barrier.

However, all plants in all plots were infected by 32 days after planting. At this time plants all had symptoms of severe mottle and leaf distortion and fruit failed to develop. At 14 days after planting, 1-5 winged and non-winged aphids were present on each zucchini plant, these were identified as melon aphid (*Aphis gossypii).*

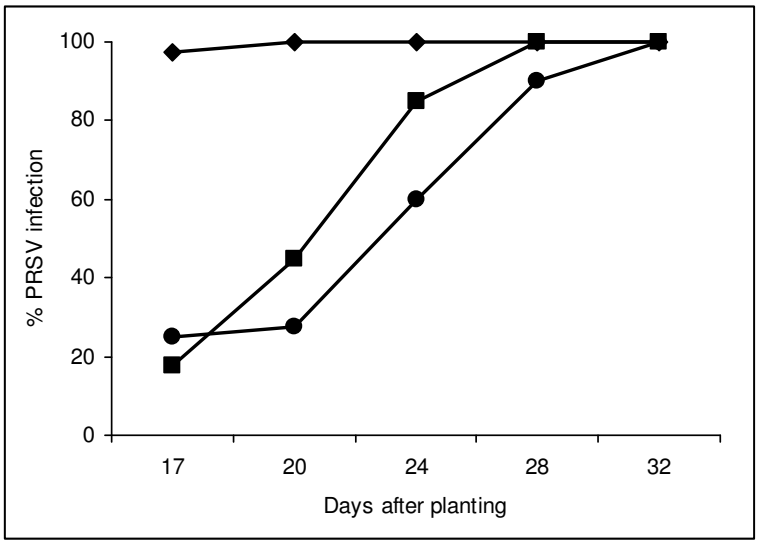

Fig. 2. Pathogen progress curves for zucchini plants infected with PRSV in plots at 1m and 15 m distances from a 100% infected virus source in trial 2; ♦ = 1m from virus source; ■ = 15m from virus source without shade-cloth barrier; ● = 15 m from virus source with shade-cloth barrier.

Discussion

When small plots of zucchini separated by fallow were planted 1m and 15 m away from a PRSV source, the virus spread faster to plots next to this source than to those further away, but there was no effect of wind direction even though the wind direction is from east to west at the site during summer. Spread to the plots 15m away developed more slowly initially but caught up later as all plants in both trials became infected by 32-35 days after planting regardless of plot distance from the source. Aphids were on all plants by 14 days after planting and presence of a shade-cloth acting as a physical barrier to aphid movement had only a marginal effect in delaying virus spread. These trials were done when peak aphid populations were present and this was reflected in the rapid virus spread that occurred.

Harvest of zucchini fruit usually starts four weeks after planting and, when the plants are infected prior to flowering all fruit is usually unmarketable due to quality defects. Deployment of a non-host barrier crop instead might have been more effective than the physical barrier used here. This is because a non-host barrier would allow

migrant aphids to probe the non-host plant and thereby lose the virus from their mouthparts before moving onto probe susceptible crop plants.

References

Coutts BA, Jones RAC (2005) Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. Aust J Agric Res 56:847-858.

Coutts BA, Kehoe MA, Jones RAC (2011) Minimising losses caused by Zucchini yellow mosaic virus in vegetable cucurbit crops in tropical, sub-tropical and Mediterranean environments through cultural methods and host resistance. Virus Research (in press)

Coutts BA, Thomas-Carroll ML, Jones RAC (2004) Patterns of spread of Tomato spotted wilt virus in field crops of lettuce and pepper: spatial dynamics and validation of control measures. Ann Appl Biol 145:231-245.

Greber RS, Persley DM, Herrington ME (1988) Some characteristics of Australian isolates of zucchini yellow mosiac virus. Aust J Agric Res 39:1085-1094.

Herrington ME (1987) Yield and quality of Cucurbita maxima increase with delayed infection by papaya ringspot virus type W. Qld J Agric Animal Sc 44:117-121.

Jones RAC, Smith LJ, Gajda BE, Latham LJ (2005) Patterns of spread of Carrot virus Y in carrot plantings and validation of control measures. Ann Appl Biol 147:57-67.

McLean GD, Burt JR, Thomas DW, Sproul AN (1982) The use of reflective mulch to reduce the incidence of watermelon mosaic virus in Western Australia. Crop Protection 1: 491-496.

Persley D, Horlock C (2003) Management of virus diseases and bacterial blotch of melons – HAL Project VX99037. Final report to Horticulture Australia Ltd, Queensland Department of Primary Industries, Indooroopilly, Qld.

Persley D, Cooke T, House S (2010) Diseases of vegetable crops in Australia. CSIRO Publishing, Collingwood, Vic.

Contact transmission of cucurbit viruses

Brenda Coutts, Monica Kehoe and Roger Jones. Department of Agriculture and Food, Western Australia.

Introduction

Plant viruses can be transmitted by a number of different methods, including vegetative propagation of infected plant material, by vectors such as insects, fungi or nematodes, through seed from infected plants and by contact of infective sap with wounded leaves. Viruses known to be readily contact transmitted usually have very stable virus particles that reach very high concentrations within the cells of infected plants. Most examples of such viruses are in the genera *Carlavirus, Sobemovirus Tobamovirus* and *Potexvirus*. These viruses spread (i) directly from infected plant to healthy plants when the leaves rub together under the influence of wind and (ii) indirectly when infective sap contaminating clothes, hands, cutting/pruning tools and equipment comes into contact with plants (Astier *et al* 2007).

The principal viruses infecting cucurbit crops in Australia are *Papaya ringspot virus* –type W (PRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV), all from the genus Potyvirus. These three viruses are non-persistently aphid transmitted with *Myzus persicae* (green peach aphid) and *Aphis gossypii* (melon aphid) the most important vectors. *Squash mosaic virus* (SqMV) a *Comovirus* transmitted by seed, and various beetle vectors is occasionally found infecting cucurbits in Australia (Greber 1969; Persley and Horlock 2003; Coutts and Jones 2005; Persley *et al* 2010). Potyviruses are not usually considered to be contact transmitted, but there is some anecdotal evidence suggesting ZYMV may be contact transmitted within infected cucurbit crops. Fletcher *et al*. (2002) suggested ZYMV spread in a cucurbit crop was by machinery as the disease symptoms were prevalent along the machinery tracks. There are also reports that SqMV can be transmitted during pruning or harvesting (Blancard *et al.* 2005).

From understanding of the mechanisms by which a virus spreads this information can be used to improve to integrated virus disease management approaches towards effective control. This study describes a series of glasshouse experiments undertaken to determine if ZYMV and SqMV can be transmitted by rubbing or crushing infected leaves onto healthy plants, and if blades contaminated with infective sap will transmit these viruses.

Materials and Methods

The virus isolates used in all experiments were ZYMV Knx-1 and SqMV Bme-1 maintained in zucchini cv. Blackjack. All experiments were done in insect-proofed glasshouse using zucchini cv. Blackjack. Leaf samples were tested for virus infection by ELISA as described by Coutts and Jones (2005). The polyclonal antiserum to ZYMV was from Prime Diagnostics, Netherlands and for SqMV was from Loewe Biochemica.

Experiments 1a-c, were done to determine if ZYMV and SqMV could spread from an infected to a healthy plant by leaf to leaf contact. For each experiment, a leaf from an infected zucchini plant (ZYMV and SqMV Expt 1a, ZYMV only 1b and c) was rubbed on the surface of a healthy zucchini leaf. For Expt 1a, b and c, five, six or ten plants were rubbed with infected leaves, respectively. At 20-21 days after rubbing tip leaves were sampled from each plant and tested by ELISA.

Experiments 2a-c, were done to simulate virus transmission by leaf crushing such as when leaves are damaged by machinery wheel tyres. A rubber mallet with parafilm secured with an elastic band to cover its head (5cm diameter) was used to tap the leaves of an infected zucchini plant (ZYMV and SqMV Expt 2a, ZYMV only 2b and c). The number of tappings was 1, 3 or 5 on the infected plant leaf followed by the same number of taps on the test plant. Five plants were used for each mallet treatment. At 24-34 days after rubber mallet inoculation tip leaves were sampled from each plant and tested by ELISA.

Experiments 3a-c, were done to simulate virus transmission on contaminated tools such as knives at cucurbit fruit harvest. A scalpel blade was used to cut through the leaf petiole (Expt 3a) or small fruit (Expt 3b) of a zucchini plant infected with ZYMV and SqMV (Expt 3a), or ZYMV only (Expt 3b). This blade was then used to cut off leaf petioles from a zucchini test plant. The number of cuts made on each infected plant were 1, 3 or 5 followed by the same number of cuts on the test plant. Five plants were used for each cutting treatment. At 34-39 days after cutting, a leaf tip was tested by ELISA.

Results

In Experiment 1a, when leaves with mixed infection with ZYMV and SqMV were rubbed onto leaves of healthy plants, most plants became infected with ZYMV (4) and SqMV (5). In Expts 1b and 1c, all 16 plants rubbed with ZYMV isolate Knx-1 became infected. None of the control plants ever became infected.

In Experiment 2a, when healthy leaves were lightly crushed (1 tap) with a rubber mallet contaminated with both ZYMV and SqMV, 1 and 3 plants became infected with ZYMV and SqMV, respectively (Table 1). When moderately crushed (3 taps), the numbers of plants infected were 2 (ZYMV) and 1 (SqMV), and when heavily crushed (5 taps) 4 plants became infected with both viruses. In Expts 2b and c, after light crushing (1 tap) none or one plants became infected this increased to three plants when moderately crushed (3 taps), and when heavily crushed (5 taps) four to five plants became infected.

In Expt 3a, no plants became infected with SqMV following cutting (1, 3 and 5 times) with a scalpel contaminated with infective sap. When 3 or 5 cuts were made one and three plants became infected with ZYMV. In Expt 3b, no plants became infected when cut once, and two plants each became infected when cut 3 or 5 times

Discussion

This study shows ZYMV can be spread readily by infected leaf-to-healthy leaf contact, and on blades contaminated with infective sap. This provides important new information on ZYMV epidemiology as before this study, excluding experimental mechanical transmission to soft plants in presence of an abrasive, the only known methods of natural ZYMV transmission were via aphid vectors and occasionally in cucurbit seed. ZYMV epidemics usually occur extremely rapidly and this may in part be due to contact transmission between plants. Cucurbit plants have leaf surfaces with long, sharp hairs that can easily cause fine scratches, so the infection probably occurs through minute wounds caused by abrasive contact between leaves of healthy and infected plants. ZYMV was also transmitted by crushing healthy leaves in presence of infective sap as may happen when plants are handled or machinery travels through a crop. Also, in simulated fruit harvesting blades contaminated with infective sap spread ZYMV.

SqMV was also transmitted by leaf-to-leaf contact and by leaf crushing but not on contaminated blades. However, further work with SqMV-infection is needed using a virus source that is free of ZYMV.

The ease with which ZYMV could be transmitted was surprising and highlights the importance of hygiene such as washing down machinery, disinfecting cutting tools and limiting handling and movement within crops. This information necessitates modification of the integrated management strategy for viruses of cucurbits to address contact transmission.

References

Astier S, Albouy J, Maury Y, Robaglia C, Lecoq H (2007) Principals of Plant Virology, genome, pathogenicity, virus ecology. Science Publishers. Enfield, NH, USA.

Blancard D, Lecoq H, Pitrat M (2005) A colour atlas of cucurbit diseases, observations, identification and control. Manson Publishing Ltd. London.

Coutts BA, Jones RAC (2005) Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. Aust J Agric Res 56:847-858.

Fletcher JD, Wallace AR, Rogers BT (2000) Potyviruses in New Zealand butternut squash (Cucurbita maxima Duch): yield and quality effects of ZYMV and WMV-2 virus infections. NZ J Crop Hort Sc 28:17-26.

Greber RS (1969) Viruses infecting cucurbits in Queensland. Qld J Agric Animal Sc 26: 145-171.

Persley D, Cooke T, House S (2010) Diseases of vegetable crops in Australia. CSIRO Publishing, Collingwood, Vic.

Persley D, Horlock C (2003) Management of virus diseases and bacterial blotch of melons – HAL Project VX99037. Final report to Horticulture Australia Ltd, Queensland Department of Primary Industries, Indooroopilly, Qld.

Investigation of viruses infecting vegetable cucurbits in Victoria

Brendan Rodoni, Denis Persley, Cherie Gambley

Introduction

Cucurbits belong to the family Cucurbitaceae and comprise frost sensitive, tendril-bearing vines growing predominantly in subtropical and tropical regions (Persley et al., 2010). The most important cultivated species in Australia are cucumber, rockmelon, watermelon, pumpkin squash and zucchini with zucchini, pumpkins and cucumbers being the most widely grown in Victoria.

Cucurbits are susceptible to a wide range of fungal bacterial and viral pathogens and parasitic nematodes. However a detailed survey of the viruses of cucurbits has not been conducted. We report here the findings of surveys conducted between 2008 – 2010 from Murray, Gippsland and Mornington Peninsula vegetable growing districts in Victoria. Surveys of cucurbit crops were conducted over several years in Victoria to accommodate the various cropping locations, variations in virus and vector activity between years and distribution of potential alternative weed hosts.

Methods

The method used for the cucurbit survey in Victoria was in line with the sampling and testing range used for similar surveys in Queensland. Accurate data on crop locations, disease incidence and symptoms, cultivars collected and crop age was collected when ever possible. Sampling consisted of a 200 random leaf samples /crop which are tested for a range of viruses in groups of 10 using ELISA. Plantings of approximately the same age on one farm can be one crop. Symptomatic plants representing the range of symptom types seen can be collected as individual additional samples.

Visual estimates of virus symptoms were made and samples collected for further analysis. At each property a 200 leaf sample was collected by ELISA for *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Papaya ringspot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV). Samples representing "plants of interest" were also collected and screened for the same viruses using ELISA. Agdia ELISA kits were used for each ELISA test unless otherwise stated. In some instances additional PCR tests were conducted on some plants.

Results and conclusions

WMV was detected at significant levels in Swan Hill but not at Wemen (only 0.5%) or in the Mildura district. The incidence of WMV on some Swan Hill properties was extremely high in both zucchini and squash crops and ranged from <1 % to 80% in some mature crops. SqMV, PRSV and ZYMV were not detected in the survey in any of the three surveys conducted. CMV was detected in a total of 4 four samples in the 2009 and 2010 surveys, however there did not appear to be any correlation between the presence of CMV and the symptoms expressed by the zucchini plants.

Paddymelon was found to be infected with WMV on several properties in different years and CMV was detected in paddymelon at property 1. These findings highlight the role this weed can play as an alternate host and a source of inoculum of cucurbit viruses.

In the second year of the surveys there was some confusion generated on the incidence of WMV in field samples due to the source of WMV antiserum used in the ELISA tests. Although frustrating it does reflect potential variation between strains of WMV. Similar experiences with WMV have been reported from the USA Vincelli and Seebold, 2009) who reported the detection of a potyvirus in symptomatic cucurbit crops using the AgDia Inc. Potyvirus group ELISA and which tested negative using the AgDia Inc. CVS test which includes tests for *Cucumber mosaic cucumovirus*, *Impatiens necrotic spot tospovirus*, *Papaya ringspot potyvirus*, *Squash mosaic comovirus*, *Tobacco mosaic tobamovirus*, *Tobacco ringspot nepovirus*, *Tomato mosaic tobamovirus*, *Tomato ringspot nepovirus*, *Tomato spotted wilt tospovirus*, *Watermelon mosaic potyvirus* (WMV) (formerly *Watermelon mosaic virus 2*) and *Zucchini yellow mosaic potyvirus*. RNA was extracted from two symptomatic Potyvirus "positive" pumpkin samples and a potyvirus was amplified using potyvirus-specific primers. Sequence analysis identified a strain of WMV with 95 – 97 % homology with multiple accessions of WMV strains. These results indicate that a strain of WMV is present in Kentucky which does not react to the current AgDia Inc. Tissue samples from the PVG-positive, CVS-negative plants analysed in this study were provided to AgDia Inc., in order to facilitate the development of a WMV-specific ELISA test that will detect this new strain.

There appears to be a poor association of the presence of WMV and symptoms expressed on zucchini plants in the field. The preferred cultivar of Zucchini was Congo and it is possible that this cultivar is more susceptible to WMV than other zucchini cultivars. Symptoms associated in Congo and which only WMV was detected include mild mosaic, mosaic, severe mosaic which included leaf deformation and plant stunting. WMV was also detected in a zucchini plant showing a golden mosaic symptom at Property 1. The zucchini cultivar Crowbar generally had a lower incidence of WMV infection (e.g. March 2010 grower 1 Block 2) and this may be an important component of an overall IPM strategy for controlling WMV losses in cucurbits.

WMV was also associated with a range of mosaic symptoms in squash including the fruit symptom referred to as "Aussie Gold". A squash plant that was showing the Aussie Gold symptom on its fruit also tested negative for WMV and none of the remaining viruses was detected in these plants. Symptom variability could partly be due to time of infection, rate of growth etc. However the observed inconsistency may be due to variation within the virus or possibly a second viral pathogen which was not detected with the diagnostic tests used in this study.

Because the correlation between the presence of WMV and symptoms on the zucchini plants was so strong it is difficult to identify additional potyviruses that may be present in the symptomatic zucchini plants as it is likely that the potyvirus specific primers are likely to detect and amplify WMV as confirmed by the sequence data. As an additional screen for other potyviruses we tested the individual samples from Property 1 for the presence of *Apium Virus Y* (ApVY). ApVY is a potyvirus that is thought to be native to Australia and infects native Apiaceae as well as carrot and celery. Interestingly this PCR did generate a band but which was not of the correct size. This amplicons was sequenced and in one isolate yielded only an 84% match with the nearest potyvirus, which happened

to be WMV. This result suggests that there is a possibility of a second potyvirus that is infecting the zucchinis and this could explain the range of virus-like symptoms that are observed in the zucchini crops in the Swan Hill area.

Based on these results from the 2009 survey, samples from weeds in the Apiaceae, particularly fennel or aniseed weed were targeted at Property 1 as this property, which is located in the Swan Hill area, consistently had more severe virus symptoms on cucurbits when compared to other zucchini growers in the district. Coincidentally Property 1 uses fennel (or aniseed weed), which is a member of the Apiacaeae as a wind break surrounding his vegetable crops. A small number of fennel samples were collected in the 2010 survey and each sample tested negative for potyviruses using RT-PCR. Despite this result the possibility that these border plants are an alternate host for potyviruses that can infect zucchinis should be examined further. More research is required in this area.

Acknowledgements

We would like to acknowledge the efforts of Matt and Brian from Swan Hill Chemicals for helping us with grower visits in the Swan Hill district and to Sally-Ann Henderson for her help in the Wemen and Mildura districts. We would also like to thank the growers who welcomed us on to their properties.

References

Persley, D, Cook, T. and House S. (2010). Diseases of vegetable crops in Australia. CSIRO Publishing. 9780643096387.

Vincelli, P., and Seebold, K. (2009). Report of a Watermelon mosaic potyvirus strain in Kentucky undetected by ELISA. Online. Plant Health Progress doi:10.1094/PHP-2009-0313-01-BR.

The effects of climate change on plant virus epidemics of vegetables in Australia
Kyla Finlay, Jo Luck, Denis Persley and Brendan Rodoni

A draft manuscript has been prepared on the effects of climate change on plant virus epidemics of vegetables in Australia.

The potential impacts of climate change on plant virus epidemics in food crops has been poorly studied. Given that the Australian vegetable industry was estimated to be worth approximately $3.1 billion dollars in 2006/07 (ABS 2008) this is a serious knowledge gap. The aim of this paper is to review potential impacts of climate change on vegetable viruses in Australia with a view to enhancing the capacity of the vegetable industry to implement integrated virus disease management programs and ultimately reduce the potential economic impact of climate change.

For Australia the best estimate for annual average temperature increases by 2030 are 1°C with slightly less (0.7° to 0.9°C) in the coastal south and north east and slightly more (1°C to 1.2°C) inland. (CSIRO & BoM 2007). It is predicted that these changes will result in an increase in daily temperature extremes, more extreme weather events and a reduction in rainfall in southern and eastern Australia. It is difficult to predict how these changes will affect plant virus epidemics in vegetable crops. Plant virus epidemics are more likely when plants are exposed to environments where the viruses have not co-evolved with the wild ancestors of those plants (Jones 2009) and climate change is likely to promote this phenomenon as changes in temperature, rainfall and frost frequency will alter the current geographic range of crops, weeds and insect vectors.

The three host/virus/vector scenarios discussed in this paper highlight how difficult it will be to predict the impact of a changing environment on plant virus disease epidemics in vegetables. It is possible for example that existing resistant cultivars could be affected as some R genes fail or are less effective under high temperatures (e.g. above mid 30s). Another possibility is that crops, particularly irrigated vegetable crops, could be more vulnerable to virus infection as they are the green islands in a dry landscape attracting migrating insect populations. The extended production cycles of many vegetable crops that will result in earlier plantings because it is warmer and there are less frosts, will reduce the length of production breaks and therefore enhance the green bridge for viruses and their insect vectors.

It is clear that cropping systems, insect vector incidence and the abundance of alternate hosts for viruses and insect vectors will change with climate change. To counteract increased losses in vegetable production it is imperative that each cropping system be carefully monitored for the emergence of new plant disease epidemics.

Discussion

The surveys for virus diseases conducted over three years provided an audit of the current health status of key vegetable crops for virus diseases.

Major crops in all important production areas were surveyed at least once during the project and key industry contacts were accessed to discuss important issues in their region or area of expertise.

These data were clearly vital before making recommendations on future needs for implementing integrated viral disease management or further research which may be needed to better understand the serious disease issues identified.

The surveys over three years in the Tasmanian vegetable processing industry provided a benchmark for the virus disease status of processing crops, particularly peas, beans and brassicas. In general, relatively low levels of virus infection were detected, suggesting that virus diseases currently have minor impact on processing crops in the State. The survey data provided valuable information for the industry, highlighting potential key viral pathogens that may require further monitoring and assist in maintaining high health and quality of processed vegetable crops in Tasmania.

Serious virus disease problems were identified in several crops in all mainland States. Vegetable cucurbits were severely affected by aphid transmitted potyviruses in Western Australia, Victoria, New South Wales and Queensland. The importance of particular viruses in cucurbits varied between States with Zucchini yellow mosaic virus (ZYMV) being dominant and very damaging in Kununurra, Carnarvon and, to a lesser extent, north Queensland. Papaya ringspot virus dominates in south Queensland where it causes extensive damage in melon and vegetable cucurbit crops and is the most common virus detected in north Queensland cucurbits. Watermelon mosaic virus was widespread and often damaging in zucchini crops in Swan Hill, Victoria.

The aphid transmitted Bean common mosaic virus caused crop failure of borlotti beans at Kununurra over two seasons. The epidemiology and management of this problem requires investigation.

Despite an outbreak of Tomato mosaic virus in capsicums at Virginia in 2009/10, Tomato spotted wilt virus is endemic in greenhouse crops and remains the most significant constraint on production. This virus is also a problem in capsicum and lettuce production in the Sydney basin.

Three viruses transmitted by whiteflies are or have the potential to be a significant factor in production. The begomovirus Tomato yellow leaf curl virus, first identified in Australia (south Queensland) in 2006, is causing crop losses of at least several million dollars annually in tomato crops at Bundaberg and threatens the major production area of Bowen/ Burdekin. All begomoviruses are transmitted by the silverleaf whitefly (B biotype). The crinivirus Beet pseudoyellows virus (BPYV), transmitted by the glasshouse whitefly, has previously been found only on dandelion in Tasmania. In this project the virus was found to be widespread in glasshouse cucumber crops in New South Wales and Virginia (SA) and causing cucumber yellows disease. The effects on production require quantification but the incidence in crops was frequently high and growers reported

reduced fruit set. The symptoms of Tomato torrado virus were first seen in greenhouse tomato crops on the North Adelaide Plain in 2004 but the cause was not identified until 2008. This virus has caused significant losses in susceptible tomato cultivars and has the potential to infect capsicum and eggplant.

In addition to benchmarking surveys, experimental work in the project provided new information on virus spread by contact, virus tolerant zucchini varieties, the cause of lettuce big vein disease and the epidemiology of some recently identified viruses.

The achievements of the project in relation to projected outcomes are discussed below. A baseline understanding of the impact of virus diseases on the vegetable industry has been provided as outlined above and in the results section. It is noteworthy that project team also identified the cause of three diseases which had been present in the industry for some years-cucumber yellows (Beet pseudoyellows virus), capsicum yellow vein (Ranunculus white mottle virus) and Tomato torrado virus. This expertise is vital in maintaining a biosecurity capacity for protection of the industry.

Project team members have previously identified and developed management plans for Capsicum chlorosis virus and Tomato yellow leaf curl virus.

An increased awareness of virus diseases and there management has been provided through industry seminars, personal contact at all levels of the vegetable industry and the publication of six reference notes which form the basis of a guide to the integrated management of virus diseases in vegetables. The industry seminars, some of which have involved training in virus identification and management, have been held in all mainland states and have targeted all areas where virus diseases are a production problem. The published material has been linked to the knowledge management system through the vegetable industry development program.

The training, information and survey data will allow growers and consultants to make more informed decisions aimed at reducing the impacts of virus diseases on crop performance and profitability. The benefit cost ratio of the project was estimated at 4.6:1 over a project life of 8 years at a discount rate of 5%. Considerable indirect benefits were also identified. Promoting a more effective and durable approach to virus disease management will take longer than one project period of three years and there will be a gradual accrual of benefits for some years to come, particularly if the processes begun in VG07128 are supported with further project work to maintain the interest, momentum and the information base.

There has been increased use of virus resistant or tolerant varieties where available and their use has been promoted as part of an integrated virus management plan. Zucchini varieties with resistance to potyviruses and tomato and capsicum with resistance to Tomato yellow leaf curl virus and Tomato spotted wilt virus are examples. There is also an urgent need to broaden the range of virus resistant cultivars available and investigate several situations where resistance genes have failed.

Disease management based on area wide management systems that reduce inoculum levels and vector populations have been promoted with both industry and funding bodies. The project has contributed to these activities in several areas, including the North

Adelaide Plain and Bundaberg. Proposals have also been prepared seeking cross industry funding to address whitefly management in cotton, grains and vegetable industries.

A discussion on the likely impacts and management of climate change were addressed in a desk top study which assessed impacts on cropping systems, viruses and their insect vectors.

Technology transfer

Providing information and capacity to allow greater adoption of integrated management of viral diseases in the vegetable industry was a key aim of the project.

The methods used to increase the awareness and adoption of integrated viral disease management included meetings/ seminars in all mainland States, the preparation of a best practice guide for virus disease management and contributions to other project outcomes within the HAL vegetable pathology program.

The meetings and seminars were held in at least once in all major vegetable production areas and information presented and discussions had on the nature and management of virus diseases in vegetables. Consultants and industry technical staff were encouraged to attend as this group was seen as critical in promoting more effective viral disease management and integrating the information provided with crop protection and production systems in specific crops and districts.

Team members successfully established productive relationships with representatives of major seed companies, crop consultants, major producers and regional agribusiness retailers to ascertain the nature of crop protection problems and provide information and assistance to minimise these.

The best practice guide on integrated viral disease management was based around five Reference notes on the nature and management of virus diseases in vegetables. Information on specific virus diseases or crops was then added to the core data. This information is available in both hard copy and electronic formats.

The reference material has been linked to the Vegetable Industry Development Program (VIDP) and uploaded to the AusVeg website. Information and publications from VG07128 and related areas made an important contribution to the Thrips and Tospovirus component of the IPM sub-program, particularly the managing thrips and tospoviruses resource developed within this program.

The Ref note on tobamoviruses was translated into Cambodian and Vietnamese to assist growers on the North Adelaide Plain and contribute to the LOTE component of the VIDP.

Several project team members of VG07128 prepared the virus management sections of the best practice production guides for brassicas and lettuce developed as part of VG07110.

The project leader of VG07128 was also the senior editor and contributing author to *Diseases of vegetable crops in Australia* which was supported through VG07136. This dual role had a significant impact on the information on virus diseases provided in the

book and the capacity to obtain good images of disease symptoms during field surveys as part of VG07128.

A list of industry seminars/ meetings/ field days is provided as appendix
A bibliography of technical articles, conference papers and media articles is provided.

Recommendations

- Measures to promote the continued adoption of integrated viral disease management as part of crop protection programs be supported
- Further work be undertaken on the causes, impact, epidemiology and management of lettuce big vein disease
- Further work on the area wide management of insect virus vectors be supported including cross industry links to address the broad host ranges of both the vectors and viruses transmitted.
- The epidemiology and management of several virus disease outbreaks requires investigation before management systems can be implemented. These include Bean common mosaic virus in beans at Kununurra and Cucumber mosaic virus in capsicum at Carnarvon.
- The expertise and experience of the project team in VG07128 continued to be supported and used by the vegetable industry to provide professional expertise in disease management and biosecurity issues.

Acknowledgements

We thank the following individuals and organisations for their assistance with disease surveys, project communications and other activities:

DEEDI Qld-David Carey, Des McGrath, Shane Dullahide, Ian Walker, Louise Morgan, Elio Jovicich
NSW Industry and Innovation- Sylvia Jelinek, Philip Wilk
Victoria Department of Primary Industries- Sally-Ann Henderson
Slobodan Vujovic Vegetable Growers Association of Victoria
Swan Hill Chemicals
Rijk Zwaan Australia-Steve Natsias, Dusanka Milunovic, Arie Beadle, Trevor Pezet, Phil Ritchie
Syngenta Seeds-Adam Thomas, Peter March, Terry Alderdice, Gerard Sippel
Hortus Bndaberg
Chris Monsour Bowen
Paul Pezzaniti CRT Virginia
SARDI South Australia-Barbara Hall, Kaye Ferguson, Sue Pederick
Moraitis-Philip McLaughlin

VG07128 Industry seminars/meetings/field days

Date	Location	Topic	Presenters	No. of attendees
7/2/2008	Virginia SA	Discussion of virus issues in protected vegetable crops	Persley, Gambley, Rodoni, Barfield	15
11/2/2008	Bowen QLD	Integrated virus disease management in vegetable crops	Persley, Gambley	20
12/2/2008	Gumlu QLD	Virus disease management in vegetable crops	Persley, Gambley	10
13/2/2008	Ayr QLD	Virus disease management in cucurbits	Persley, Gambley	40
11/3/2008	Carnarvon WA	Virus disease management in cucurbits and capsicum	Coutts	20
20/5/2008	Kemps Creek NSW	Disease management in greenhouse cucumbers	Tesoriero, Forsyth, Persley	50
27/5/2008	Bundaberg QLD	Virus disease management in capsicum and cucurbits	Persley	40
23/10/2008	Virginia SA	Grower/industry discussion of virus disease management	Persley, Gambley	10
11/11/2008	Richmond NSW	Integrated disease management in lettuce	Tesoriero, Forsyth	30
31/3/2009	Bowen QLD	Integrated viral disease management in vegetable crops	Persley	25
1/4/2009	Ayr QLD	Integrated viral disease management in vegetable crops	Persley	40
2/4/2009	Mareeba QLD	Integrated viral disease management in vegetable crops	Persley	25
6/4/2009	Perth QLD	Hort Guard meeting – biosecurity issues for cucurbits	Coutts, Jones	20
6/5/2009	Melbourne VIC – Aus Veg Convention	Integrated viral disease management project overview	Persley	150
12/5/2009	Carnarvon QLD Hort. field day	Virus diseases of vegetables	Coutts	60
30/5/2009	Virginia SA	Virus diseases in vegetable crops	Persley, Thomas, Burfield	50
7/9/2009	Bundaberg QLD	Field walk – vegetable virus diseases	Persley, Thomas, Gambley, DR, S Adkins, DR	9
Sept/Dec	Sydney basin NSW	Training sessions for growers/consultants on lettuce IPM (4 days every 2 weeks)	Tesoriero, Forsyth, McDougall	variable 10 to 30
17/3/2010	Swan Hill VIC	Virus disease management in cucurbits	Rodoni, Gambley, Persley	16
10/7/2010	Kununurra WA	Zucchini yellow mosaic virus in cucurbits	Coutts	50
9/8/2010	Bundaberg QLD	Virus management in vegetable	Persley	50

Date	Location	Topic	Presenters	No. of attendees
11/8/2010	Gympie QLD	Virus management in vegetable crops	Persley and other HAL pathology program team members	60
12/8/2010	Gatton QLD	Virus management in vegetable crops	Persley and HAL pathology program team members	40
14/9/2010	Carnarvon QLD	Management of virus and insect pests in vegetables	Kehoe, Persley, Broughton, Jones, Thomas	55
13/10/2010	Yeppoon QLD (Chem cert training course)	Plant diseases and their management	Persley	30
15/11/2010	Shepparton VIC (Morartis Produce/HFF)	Disease management in protected cropping systems	Gambley, Tesoriero, Persley, Thomas	70
1/12/2010	Virginia SA	Management of tobamovirus in greenhouse crops	Persley, Burfield	15
10/2/2011	Manjimup WA	Management of virus diseases in vegetable brassica crops	Coutts	20

Bibliography

Technical articles/ conference papers/ media releases

Technical articles

Coutts B and Kehoe M (2010) Zucchini yellow mosaic virus in cucurbit crops. Farm note. Department of Agriculture and Food Western Australia

Coutts B (2010) Cucumber mosaic virus in capsicums. Farm note. Department of Agriculture and Food Western Australia.

Dimsey R, Carey D and Henderson, SA (eds) (2010). Brassica best practice-integrated pest management. Department of Primary Industries Victoria/ DEEDI Qld.

Dimsey R, Carey D and Henderson SA (eds) (2010). Lettuce best practice-integrated pest management. Department of Primary Industries Victoria/ DEEDIQld.

Forsyth LM, McDougall S and Terras MA (2011). Managing INSV and western flower thrips. New South Wales Industry and Investment PrimeFact.

Forsyth LM, Tesoriero L and Jelinek S (2009) Healthy farms: good on-farm hygiene and sanitation. New South Wales Industry and Investment PrimeFact

Gambley CF, Thomas JE, Persley DM and Hall BH (2010). First report of Tomato torrado virus on tomato from Australia. *Plant Disease* 94, 486.

Pappu HR, Jones RAC, Jain RK (2009) Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus Research* 141, 219-236.

Persley DM and Gambley CF (2009). Virus diseases of vegetable crops in Australia. Reference note PR09/4502. DEEDI QLD

Persley DM and Gambley CF (2010) Tobamoviruses-tobacco mosaic virus, tomato mosaic and pepper mild mottle virus.Reference note PR10/5352 DEEDI

Persley DM and Gambley CF (2010) Aphid –transmitted viruses in vegetable crops. Reference note PR 10/5254. DEEDI QLD

Persley DM and Gambley CF (2011) Whitefly transmitted viruses in vegetable crops. Reference note CS0221 03/11. DEEDI QLD

Persley DM and Gambley CF (2011) Plant viruses transmitted by thrips. Reference note CS 04/11 DEEDI QLD

Van Brunschot S, Persley DM and Thomas JE (2011) Diseases caused by viroids. Reference note Cs 05/11.

Conference/workshop abstracts

Gambley CF, Persley DM, Thomas JE, Forsyth L, Tesoroiero L, Rodoni B, Coutts B, Jones R and Burfield T (2011). Virus diseases in Australian vegetable crops. 4th Conference International Working Group of Legume and Vegetable Virologists, Antequera, Spain , May 2011.

Gambley CF, Persley DM, Thomas JE, Steele V, Tesoriero L and Rodoni B (2010) Whitefly-transmitted viruses in Australian vegetable crops: endemic problems and exotic threats. 9th Australasian Plant Virology Workshop Melbourne 16-19 November 2010.

Jelinek S, Forsyth L and Tesoriero L (2011) Virus surveys of zucchini crops and alternative hosts in the Sydney basin. 18th biennial Australasian Plant Pathology Conference, Darwin, Australia. April 2011

Jones, R.A.C. (2008) Management of virus diseases in organic agriculture. Abstracts of the 9th International Congress of Plant Pathology, Turin, Italy, August 24-29, p.50

Jones, R.A.C. and Coutts B.A. (2009) Using epidemiological information to help manage tospoviruses effectively – the experience with TSWV in Australia. Abstracts of the 9th International Symposium on Thysanoptera and Tospoviruses, Queensland, Australia, 31 Aug-4 Sept 2009, p.45.

Jones R.A.C. (2009) A global view of tospoviruses. Abstracts of APSA-AVRDC Workshop on Topsoviruses and thrips vectors, Bangkok, Thailand, 8 November 2009.

Kehoe M, Coutts B, Wylie S, Webster C and Jones RAC (2010) Sequence diversity of Australian *Zucchini yellow mosaic virus* isolates. Abstracts of 9th Australasian Plant Virology Workshop, Melbourne, Australia, 16-19 November 2010. p. 4.

Lambert S, Dann A, Wilson C (2010) Virus incidence within processing vegetable crops in Tasmania, and discovery of a putative novel luteovirus. 9th Australasian Plant Virology Workshop, Melbourne 16-19 November 2010.

McMichael LA, Coleman E, Spence D and Persley DM (2008) Detection and characterisation of viruses from sweetpotato in Papua New Guinea and Queensland, Australia. 8th Australasian Plant Pathology Workshop, Rotorua, New Zealand, November 2008.

Persley DM, McGrath D, Sharman M and Walker I (2009) Breeding for tospovirus resistance in a package of disease resistances for capsicum and tomato. IX International Symposium on Thysanoptera and Tospoviruses, September 2009, Gold Coast Queensland, Australia.

Persley DM, Gambley CF, Thomas JE, Steele V (2010) Current and potential viral disease issues for the Australian vegetable industry. 9th Australasian Plant Virology Workshop Melbourne November 2010.

Persley DM, Sharman M, Thomas JE, Gambley CF (2010) Alternative hosts of two tospoviruses in Queensland, Australia. 11th International Plant Virus Epidemiology Symposium June 20-24, Cornell University, Ithaca, New York

Terras MA, Chambers G, Forsyth LM and Tesoreiro LA (2011) Detection and eradication of *Impatiens necrotic spot virus* on ornamental nursery stock in New South Wales. 18th biennial Australasian Plant Pathology Conference, Darwin, Australia. April 2011.

Tesoriero L, Forsyth L, Persley,D and Gambley C (2010). Emerging disease threats to protected cropping vegetable and ornamentals. Abstracts Global Biosecurity Conference 2010, 28 February-3 March Brisbane, p78.

Tesoriero L, Forsyth LM and Terras MA (2009) New and emerging disease threats to the Australian greenhouse and ornamental industry. Australian Hydroponic Greenhouse Association Conference, Sydney, Australia.

Grower publications/ media releases/ news items

Coutts B (2009) Virus diseases in vegetable crops on the Swan Coastal Plain. In: Vegetables WA – Good practice guide

Coutts B (2009) Understanding a devastating virus disease of cucurbits. WA Grower December 2009, p13

Coutts B (2009) Slowing the spread of virus into cucurbit crops. AgMemo, December 2009, Kununurra

Coutts B (2009) Virus resistant varieties. AgMemo, June 2009, Carnarvon.

Coutts B (2010) Virus diseases in vegetable crops. AgFlash June 2010, Carnarvon

Coutts B (2010) Aphids and virus diseases in Kununurra. ABC rural report, November 2010

Coutts B (2010) Virus disease in vegetable crops at Carnarvon. ABC rural report, December 2010.

Appendices

Appendix 1

Incidence and distribution of viruses in vegetable plantings in Western Australia

Brenda Coutts, Monica Kehoe and Roger Jones. Department of Agriculture and Food, Western Australia.

The major vegetable growing areas in Western Australia are located in the temperate south-west (especially Manjimup, Gingin and Carabooda), sub tropical mid north (Carnarvon) and tropical north (Kununurra) of the state. The total value of vegetable production in these areas is more than $250 million annually with an area of production of more than 7300 ha and a total volume of 230 000 tonnes (ABS 2009). A wide variety of crops are grown mostly for the domestic market but also some for export. Key crops include vegetable brassicas, other leafy vegetables, cucurbits, fruiting vegetables, root and tuber crops, and others.

Previous surveys for virus diseases infecting vegetables in Western Australia have found *Beet western yellow virus* (BWYV), *Bean common mosaic virus* (BCMV), *Capsicum chlorosis virus* (CaCV), *Carrot virus Y* (CarVY), *Celery mosaic virus* (CeMV), *Cucumber mosaic virus* (CMV), *Lettuce big vein disease* (LBVD), *Lettuce necrotic yellows virus* (LNYV), *Papaya ringspot mosaic virus* (PRSV), *Tomato spotted wilt virus* (TSWV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) (e.g., Coutts and Jones 2005; Latham and Jones 1997, 2003; Latham *et al.* 2003, 2004a,b; Saqib *et al*. 2005). Additional viruses have been found in surveys of potato and sweet potato crops (Wilson and Jones 1990; Tairo *et al*. 2008). However, many of these surveys were done prior to 2004 and therefore updated information on current incidence and distribution is necessary to determine the potential yield and financial losses that are occurring currently from virus diseases as well as identify if they have spread to new areas and crops.

Over a 4 year period from 2008 to 2011, vegetable crops from the three major growing areas (south-west, Carnarvon, Kununurra) were surveyed for viruses. Random and samples with virus-like symptoms were collected and tested by ELISA for a number of different viruses depending on the host crop sampled.

Overall, a total of 15,625 samples from 28 different vegetable crop types were tested from 48 properties at Carnarvon, Kununurra and in 13 areas in the south-west

(Table 1). The most commonly detect viruses were CMV, TSWV and ZYMV. The other viruses detected were BCMV, BWYV, CarVY, CaCV and LBVD.

At Carnarvon, 8,403 samples belonging to 12 crop types from 13 properties were tested. ZYMV was detected in cucumber, pumpkin, squash, watermelon and zucchini crops with incidences of up to 21% at the time of surveying. At the end of the growing seasons in 2009 and 2010 most of these crop types were 100% infected with ZYMV and crops were being abandoned as yields were very low and fruit was unmarketable. In 2010, CMV and TSWV were both detected in capsicum, chilli and eggplant crops at incidences of up to 100% and 80% for CMV and TSWV, respectively. Many crops were abandoned as the fruit was unmarketable and yields low. Widespread TSWV infection reflected the widespread invasion of Carnarvon by western flower thrips.

At Kununurra, 2,117 samples belonging to 19 crop types from 5 properties were tested. ZYMV was detected in cucumber, squash and zucchini crops with incidences up to 100%. In 2009 and 2010 many pumpkin and melon crops were abandoned and ploughed in due to virus incidences of up to 100% prior to flowering (July/August). Large aphid flights at this time lead to rapid spread of ZYMV across most crops in the Kununurra area and complete yield loss. Crops of borlotti beans were 100% infected by BCMV in 2009 and 2010, and many crops were destroyed as no yield would be obtained. CaCV was detected in one capsicum crop and *Cauliflower mosaic virus* (CaMV) in one crop of cauliflower.

From the growing areas in the south-west, 5,105 samples from 16 crop types from 30 properties were tested. TSWV was detected in capsicum, celery, chilli, lettuce and tomato crops with incidences up to 50%. Most tomato and capsicum growers were planting TSWV-resistant varieties. In 2010, two crops of TSWV resistant varieties were found infected with TSWV, one capsicum cv. Zamboni crop grown in a shadehouse was 50% infected and one tomato cv. Swanson crop grown in the field was <1% infected. *Mirafiori lettuce virus* and *Lettuce big vein virus* that are associated in mixed infection with lettuce big-vein disease were found in 46% of lettuce crops tested, with in crop incidences of up to 100%. Crops of cauliflower and broccoli at Manjimup were infected with BWYV in December 2010, many crops were being abandoned at this time due to reduced head size and distortion. This epidemic appeared to be due to large aphid flights in October and November, presence of virus infected wild radish weeds nearby and lack of insecticide applied to seedlings prior to planting. In addition, CMV was detected in celery (3%) and tomato (<1%), and CarVY was found in a carrot crop (<1%).

In Western Australia, virus diseases continue to cause extensive yield losses in vegetable crops. Of greatest importance are ZYMV in cucurbits in Carnarvon and Kununurra, TSWV in capsicum and tomato in Carnarvon, CMV in capsicum in Carnarvon, LBVD in lettuce in the south-west, and BCMV in borlotti bean in Kununurra. Epidemics in cucurbits at Kununurra and Carnarvon were often so severe that diseased crops were abandoned. Capsicum and tomato crops are also suffering increasingly in Carnarvon. Detection of two resistance TSWV breaking strains in the south-west is cause for concern to tomato and capsicum production.

Table 1. Distribution and incidence of viruses in different types of vegetable crops in south-west, Carnarvon and Kununurra of Western Australia

% virus incidence detected (range of virus incidences within individual crop)

Type of crop	No. of properties	No. of samples tested	Potyvirus	ZYMV	CMV	Tospovirus/ TSWV	PVY	LBVV MLBVV	BWYV	TuMV	CaMV	SqMV	CaCV
South-west													
Baby Spinach	1	100	-	nt	-	-	nt	nt	-	-	-	nt	nt
Beetroot	1	100	-	nt	-	nt	nt	nt	-	nt	nt	nt	nt
broccoli	8	939	-	nt	-	nt	nt	nt	0.3 (100)	-	-	nt	nt
Cabbage	2	125	-	nt	-	nt	nt	nt	-	-	-	nt	nt
Capsicum	5	77	-	nt	-	-	-	nt	nt	nt	nt	nt	nt
Carrot	2	101	<1+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Cauliflower	8	985	-	nt	nt	nt	nt	nt	0.1 (4)	-	-	nt	nt
Celery	2	268	-	nt	3	<1	nt	nt	nt	nt	nt	nt	nt
Chilli	3	31	-	nt	-	45	-	nt	nt	nt	nt	nt	nt
Eggplant	2	43	-	nt	-	-	-	nt	nt	nt	nt	nt	nt
Garlic	1	2	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Lettuce	7	1420	-	nt	-	1(2-8)	nt	40 (12-100)	nt	nt	nt	nt	nt
Spinach	1	50	-	nt	-	-	nt	nt	nt	nt	nt	nt	nt
Tomato	5	739	-	nt	<1	2 (5-10)	nt	nt	nt	nt	nt	nt	nt
Zucchini	1	30	-	nt	-	nt	nt	nt	nt	nt	nt	nt	nt
Total	**30**	**5105**											
Kununurra													
Bean	1	360	-	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Bok Choy	1	50	-	nt	nt	nt	nt	nt	-	nt	-	nt	nt
Borlotti bean	1	75	100 (100)*	-	nt	nt	nt	nt	nt	nt	nt	nt	nt
Broccoli	1	50	-	nt	nt	nt	nt	nt	-	nt	-	nt	nt
cabbage	1	80	-	nt	nt	nt	nt	nt	-	nt	5 (5)	nt	nt
Capsicum	2	233	nt	nt	-	-	-	nt	nt	nt	nt	nt	0.4(0.4)

Cauliflower	2	81	-	nt	nt	nt	nt	nt	-	nt	-	nt	nt
Cucumber	1	20	100	100 (100)	nt	nt	nt	nt	nt	nt	nt	-	nt
Eggplant	1	51	-	nt	-	-	-	nt	nt	nt	nt	Nt	nt
English Spinach	1	40	-	nt	-	nt	nt	nt	-	nt	-	nt	nt
Pumpkin	1	50	-	nt	-	nt	nt	nt	nt	nt	nt	-	nt
Lettuce	3	290	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
Red kidney bean	1	100	1 (1)*	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Rocket	1	50	-	nt	-	nt	nt	nt	-	nt	-	nt	nt
Silverbeet	1	40	-	nt	-	nt	nt	nt	-	nt	-	nt	nt
Squash	3	231	1	1(100)	-	nt	nt	nt	nt	nt	nt	nt	nt
Tomato	3	170	-	nt	-	-	-	nt	nt	nt	nt	nt	nt
Zucchini	3	321	2	2 (<1-3)	-	nt	nt	nt	nt	nt	nt	-	nt
Total	**5**	**2117**											
Carnarvon													
Beans	3	162	-	nt	-	nt	nt	nt	nt	nt	nt	nt	nt
Capsicum	8	827	-	nt	6 (<1- 100)	3 (1- 80)	-	nt	nt	nt	nt	nt	-
Chilli	4	186	-	-	15 (11-70)	-	-	nt	nt	nt	nt	nt	-
Cucumber	5	464	6	6 (3-21)	-	nt	nt	nt	nt	nt	nt	-	nt
Eggplant	4	536	-	-	0.4 (0.7)	4 (80)	-	nt	nt	nt	nt	nt	nt
Garlic	2	4	+	nt	-	-	-	nt	nt	nt	nt	nt	nt
Pumpkin	5	878	1	1 (<1- 4)	-	nt	nt	nt	nt	nt	nt	-	nt
Rockmelon	2	132	<1	<1 (<1)	nt	nt	nt	nt	nt	nt	nt	nt	nt
Squash	2	182	15	15 (5-90)	-	nt	nt	nt	nt	nt	nt	-	nt
Tomato	2	163	-	nt	-	-	-	nt	nt	nt	nt	nt	nt
Watermelon	3	308	4	4 (<1-4)	nt	nt	nt	nt	nt	nt	nt	-	nt
Zucchini	5	719	2	2 (<1-40)	<1 (<1)	nt	nt	nt	nt	nt	nt	-	nt
Total	**13**	**8403**											

Viruses tested by ELISA, ZYMV, *Zucchini yellow mosaic virus*; CMV, *Cucumber mosaic virus*; TSWV, *Tomato spotted wilt virus*; PVY, *Potato virus Y*; BWYV, *Beet western yellows virus*; TuMV, *Turnip mosaic virus*; CaMV, *Cauliflower mosaic virus*; SqMV, *Squash mosaic virus*; CaCV, *Capsicum chlorosis virus*. nt = not tested, - = virus not detected.

References

Coutts BA, Jones RAC (2005) Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. Aust J Agric Res 56:847-858.Jones, R.A.C. and Sharman, M. (2005). Capsicum chlorosis virus infecting Capsicum annuum in the East Kimberley region of Western Australia. Australasian Plant Pathology 34, 397-399.

Latham LJ, Jones RAC (1997) Occurrence of tomato spotted wilt tospovirus in native flora, weeds and horticultural crops. Aust J Agric Res 48: 359-69.

Latham LJ, Jones RAC (2003) Incidence of Celery mosaic virus in celery crops in sout-west Australia and its management using a 'celery free period'. Aust Plant Path 32:527-531.

Latham LJ, Jones RAC, McKirdy SJ (2004) Lettuce big-vein disease: sources, patterns of spread and losses. Aust J Agric Res 55:125-130.

Latham LJ, Smith LJ, Jones RAC (2003) Incidence of three virus in vegetable brassica plantings and associated wild radish weeds in south-west Australia. Aust Plant Path 32:387-391.

Latham LJ, Traicevski V, Persley DM, Wilson CR, Tesoriero L, Coles R, Jones RAC (2004) Distribution and incidence of Carrot Virus Y in Australia. Aust Plant Path 33:83-86.

Saqib M, Jones RAC, Cayford B, Jones MGK (2005) First report of Bean common mosaic virus in Western Australia. Plant Pathology 54, 563.

Tairo F, Jones RAC, Valkonen JPT (2006) Phytoplasma from little leaf disease affected sweetpotato in Western Australia: detection and phylogeny. Annals of Applied Biology 149, 9-14.

Tairo F, Jones RAC, Valkonen JPT (2006) Potyvirus complexes in sweet potato: occurrence in Australia, serological and molecular resolution and analysis of the Sweet potato virus 2 (SPV2) component. Plant Disease 90, 1120-1128.

Wilson CR, Jones RAC (1990) Virus content of seed potato stocks produced in a unique seed potato production scheme. Annals of Applied Biology 116: 103-109.

Appendix 2

Final report New South Wales VG0 7128

Leanne Forsyth and Len Tesoriero

New South Wales Department of Industry and Innovation

Materials and Methods

Surveys and diagnostic samples

Zucchini

Over a two year period in summer seven farms were extensively surveyed in the Sydney basin for zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus II (WMVII) and papaya ringspot virus (PRSV). Samples were taken from the zucchini fields using a stratified random sampling method to collect either 200 or 300 individual samples per field. Additional samples of zucchini were taken to deliberately select symptomatic plants. Weeds and volunteer plants were sampled from within the crop and the headland areas surrounding the fields. When possible samples of 30 individual plants were taken per weed species. All samples were placed in appropriately labelled plastic bags for processing back in the laboratory. To reduce costs samples were bulk analysed in groups of 10 individual samples of the plant crop or groups of five individual samples of the weed species. To ensure equal amounts of leaf tissue of each of the 10 individual samples a leaf corer was used. Virus detection was undertaken by ELISA using the Agdia (Elkhart, USA) ZYMV, WMVII and PRSV antisera as per manufacturer's instructions. Positive controls of ZYMV, PRSV sand WMVII supplied by Agdia were used, negative controls of uninfected cucumber leaves were used. Samples were assessed as being positive or negative by having an absorbance higher than double the negative control after two hours incubation with the appropriate substrate. Virus incidence was determined based on the formula from Moran *et al.* (1983) and confidence limits and interpolation from Rolf and Sokal (1969).

To further confirm new weed species as hosts and to clarify ambiguous positives RT-PCR and sequencing was used. RNA was extracted from the sample using RNeasy (Qiagen, Valencia, USA) as per manufacturer's instructions. RT-PCR was undertaken using degenerate potyvirus coat protein primers (Pot2 and Poty1) (Colinet and Kummert, 1993) and superscript III (Invitrogen, Carlsvad, USA) as per the manufacturer's instructions. PCR product was prepared for cloning using a clean up

kit (Ultraclean PCR, Mo Bio, Solana Beach, USA) as per manufacturers instructions. PCR product was cloned into pGEM-T (Promega, Madison, USA) and transformed into *E. coli* as per manufacturers instructions. Transformed *E. coli* were grown on LB agar amended with ampicillin and X-gal at 37^{o}C overnight. Large white *E. coli* colonies were checked for plasmid inserts using PCR. PCR products were examined electrophoretically on agarose gels stained with ethidium bromide. Excess primer and salts were removed from the end PCR product using a clean up kit (Ultraclean PCR, Mo Bio, Solana Beach, CA, USA), as per the manufacturer's instructions. Sequencing reactions, in the forward and reverse directions were performed on the cleaned PCR product using BigDye3 (Applied Biosystems, Foster City, USA) as per the manufacturer's instructions.

The forward and reverse sequencing reactions of the PCR products were aligned and corrected manually using the sequence editor and aligner available in BioEdit version 7.0.5.3 (Hall, 1999). The resulting gene sequences for the different samples were aligned using BioEdit and compared to fungal isolates from the public database, using BLAST (Altschul *et al*., 1990).

Lettuce and field brassicas

Over a two year period four farms were surveyed in the Sydney basin and two from the Bathurst region for turnip mosaic virus (TuMV). Samples were taken from the field vegetables using a stratified random sampling method to collect 200 individual samples per field. Additional samples of the vegetable crops were taken to deliberately select symptomatic plants. Weeds and volunteer plants were sampled from within the crop and the headland areas surrounding the fields. When possible, amples of 30 individual plants were collected per weed species to assess weedy alternative virus hosts. All samples were placed in appropriately labelled plastic bags for processing back in the laboratory. To reduce costs samples were bulk analysed in groups of 10 individual samples of the plant crop or groups of five individual samples of the weed species. To ensure equal amounts of leaf tissue of each of the 10 individual samples a leaf corer was used. Virus detection was undertaken by ELISA using the Agdia TuMV antisera as per manufacturer's instructions. A positive control of TuMV supplied by Agdia was used, negative controls of uninfected lettuce, cabbage and broccoli were used. Samples were assessed as being positive or negative by having an absorbance higher than double the negative control after two hours incubation with the appropriate substrate. Virus incidence was determined based on the formula from Moran *et al.* (1983) and confidence limits and interpolation from Rolf and Sokal (1969).

Asian vegetables

In 2008 one Asian vegetable farm was surveyed for viral problems within the cucurbits (hairy melon,). Samples were taken using a stratified random sampling method to collect 200 individual samples per field. All samples were placed in appropriately labelled plastic bags for processing back in the laboratory. To reduce costs samples were bulk analysed in groups of 10 individual samples of the plant crop. To ensure equal amounts of leaf tissue of each of the 10 individual samples a leaf corer was used. Virus detection was undertaken by ELISA using the Agdia ZYMV, WMVII and PRSV antisera as previously described.

In 2008 a number of coriander plants were found as having poty viroid particles by electron microscopy within the RIRDC project (DAN233J Integrated management strategies for diseases and pests of Asian vegetables). To identify and clarify this emerging virus problem RT-PCR and sequencing was used as previously described.

Ornamental and emerging virus issues

In early 2010 a Spathiphyllum plant sample was sent into the plant health diagnostic laboratory in NSW. It was identified as having impatiens necrotic spot virus (INSV) by Agdia immunostrip. To further understand the spread of this virus a specific INSV RT-PCR test was adapted and surveillance was undertaken of the original infected property, nearby properties and other ornamental wholesale and retail nurseries.

Initial molecular work was undertaken using the degenerate Tospo sRNA primers. RNA was extracted from the sample using RNeasy (Qiagen) as per manufacturer's instructions. RT-PCR was undertaken using random hexamer primers and superscript III (Invitrogen) as per the manufacturer's instructions. PCR was undertaken using the degenerate Tospo sRNA primers GL3637 and GL4435c (Chu *et al.,* 2001). To ensure that RT-PCR inhibitors were not present in the sample an additional housekeeping PCR was undertaken using the primers AtrophaNad2.1a and AtropaNad2.2b for the NADH dehydrogenase ND2 subunit (Constable *et al*., 2010). PCR product was prepared for direct sequencing using a clean up kit (Ultraclean PCR, Mo Bio) as per manufacturers instructions. Sequencing reactions, in the forward and reverse directions were performed on the cleaned PCR product using BigDye3 (Applied Biosystems) as per the manufacturer's instructions.

The forward and reverse sequencing reactions of the PCR products were aligned and corrected manually using the sequence editor and aligner available in BioEdit version 7.0.5.3 (Hall, 1999). The resulting gene sequences for the different samples were aligned using BioEdit and compared to fungal isolates from the public database, using BLAST (Altschul *et al*., 1990).

An improved molecular test was developed using primers modified by Murray Sharman (adapted from Mumford *et al.* 1996) (INSV sRNA 2061F: CCAAAATCAATAGTAGCATTA; and INSV S2 CTTCCTCAAGAATAGGCA). These primers were checked for cross specificity with other tospoviruses present in Australia: tomato spotted wilt virus (TSWV); iris yellow spot virus (IYSV); and capsicum chlorosis virus (CaCV).

Samples were taken from the original infected property using a stratified random sampling method to collect 300 individual plant samples per plant type. Additional samples of plants were taken to deliberately select symptomatic plants. Weeds and any other INSV host plant were sampled from within the polyhouses, the shade houses and the headland areas surrounding the property. When possible, samples of 30 individual plants were taken per plant type. All samples were placed in appropriately labelled plastic bags for processing back in the laboratory. To reduce costs samples were bulk analysed in groups of 10 individual samples of the plant crop or groups of five individual samples of the weed species. To ensure equal amounts of leaf tissue of each of the 10 individual samples a leaf corer was used. Suspicious samples were collected from surrounding properties and other ornamental wholesale and retail nurseries. All samples were analysed using RT-PCR. Samples which tested

negative to the INSV RT-PCR and the housekeeping gene RT-PCR were reextracted and reassessed. Virus incidence was determined based on the formula from Moran *et al.* (1983) and confidence limits and interpolation from Rolf and Sokal (1969).

Glasshouse experimental work

Glasshouse host range experiments were undertaken to further understand the results identified within the zucchini survey. Seeds were collected from broad bean, wild fennel, dock and fleabane plants which appeared healthy. Collected seed and zucchini seeds were germinated in sterile UC potting mix in seedling trays within a clean glasshouse. Once seedlings were approximately one month old they were potted into pots (200mm diameter, two seedlings per pot) and mechanically inoculated with relatively fresh infected zucchini leaf tissue. The zucchini leaf tissue was collected from an infected field and placed at 4^{o}C while it was screened for the presence of ZYMV, PRSV and WMV using ELISA (as previously described). The maximum length of time between sampling the leaf tissue from the field and inoculation onto seedlings was three days. Seedlings were inoculated with zucchini infected with ZYMV, PRSV or ZYMV and WMV. Control mock inoculations were also performed. Glasshouse temperatures ranged from 14-35^{o}C, plants were watered daily and fertilized weekly. Plants were assessed weekly for visual symptoms and grown on for six weeks before being assessed for virus incidence using ELISA or RT-PCR (as previously described).

Further host range testing was undertaken on dock and fleabane using live aphid transmission. Collected dock, fleabane and commercial zucchini seeds were germinated in sterile UC potting mix in seedling trays within a clean glasshouse. Once seedlings were approximately one month old they were potted into pots (200mm diameter, two seedlings per pot) and placed into insect cages. To prepare insect vectors clean populations of *Aphis gossypii* cotton aphid (CA) and *Myzus persicae* green peach aphid (GPA) were placed onto positive virus infected zucchini plants to feed. Transfer of the two different newly infective aphid vectors was done by paintbrush from the infected zucchini plants onto the weed pot within the insect cage. Aphids were later killed using Confidor and plants were removed from the insect cages. Plants were assessed weekly for visual symptoms and grown on for six weeks before being assessed for virus incidence using ELISA or RT-PCR (as previously described). The source zucchini plants were collected from an infected field and screened for the presence of ZYMV, PRSV and WMV using ELISA (as previously described). Three different virus combinations were used: PRSV, WMV and PRSV/ZYMV/WMV.

Results and discussion

Surveys and diagnostic samples

Zucchini

Within the seven farms regularly surveyed around western Sydney there was considerable variation in virus incidence, and virus dominance between surveyed farms and throughout the growing season. Typically virus incidence decreased from a peak in February –March through to May. This change in virus incidence maybe

related to a decrease in insect vector activity, as aphid populations are higher in warmer summer periods.

Overall incidence levels of ZYMV decreased over time (it should be noted that this is over multiple zucchini crops). Farms surveyed in February had estimated levels of ZYMV ranging from 1.612% (0.904-6.536% 95% confidence limits) through to 100% (19.4%-100% 95% confidence limits). In May estimated levels of ZYMV had typically been reduced to 0% (95% confidence) to 1.421% (0.384-3.606 95% confidence limits). This change in ZYMV virus incidence maybe linked with a change in zucchini cultivars grown in the late season or with a change in aphid activity.

Levels of WMV varied highly from farm to farm in February, however by May incidence of WMV was low. In February estimated levels of WMV ranged from 2.836% (0.904-6.536% 95% confidence limits) through to 100% (19.4%-100% 95% confidence limits). In May estimated levels of WMV ranged from 2.622% (1.039-5.439% 95% confidence limits) through to 3.504% (1.581-6.585%). It should be noted that one farm had an estimated 100% virus incidence in both ZYMV and WMV early in the growing season (February). This farm consistently used knives to cut fruit off the plants. Work on virus transmission has shown that ZYMV can be transmitted on cutting knives (B Coutts pers comm..).

In contrast to WMV and ZYMV levels of PRSV increased across the growing season. Initial levels of PRSV on farms surveyed ranged from 0% (95% confidence limit) through to 1.612% (0.326-4.657% 95% confidence limits). During May levels of PRSV were typically above 5% with one farm having an estimated 100% infection (19/4-100% 95% confidence limit). One hypothesis for the initial low levels of PRSV in February maybe that there a few alternative hosts, to date no alternative hosts outside the Cucurbitaceae have been described apart from papaya. It maybe that due to the few alternative hosts, the initial inoculum pool for aphids to feed on to become infectious is small.

The alternative weed host survey associated with the zucchini crop surveys has revealed several weed host plants for growers to beware. The initial ELISA results suggested a number of alternative hosts for ZYMV, WMV and PRSV (table 1). Molecular work was carried out to confirm the presence of viruses within white clover, dock, sow thistle, fleabane and artichoke. The white clover was found to have clover yellow vein virus. None of the 10 plasmids sequenced contained ZYMV, WMV or PRSV. The artichoke was found to contain WMV and ZYMV but not PRSV based on the sequencing of 5 different bacterial plasmids. It appears that some virus strains are cross reacting with the Agdia PRSV antisera. The fleabane and sow thistle were found to contain PRSV by sequencing of 3 different bacterial plasmids. The symptoms on the fleabane were small chorotic spots, while on the sowthistle the symptoms were necrotic spots and small leaves. The farm that the fleabane and sowthistle containing PRSV were collected from was the one farm that had the highest PRSV incidence within the zucchini crops surveyed early in the season (1.612%) and the highest PRSV incidence in the late season in May (100%).

Table 1. Alternative hosts surveyed for presence of ZYMV, WMVII and PRSV by ELISA.

Common plant name	Botanical name	Virus detected by ELISA		
		ZYMV	WMVII	PRSV
Purpletop verbena	*Verbena bonanensis*	Positive	Positive	Negative
Dock	*Rumex obtusifolius*	Negative	Positive	Positive
Sow thistle	*Sonchus oleraceus*	Negative	Negative	Positive
Artichoke	*Cynara scolymus*	Positive	Negative	Positive
White clover	*Trifolium repens*	Weakly positive	Weakly positive	Weakly positive
Fleabane	*Conyza sp.*	Negative	Positive	Positive
Amaranth	*Amaranthus sp.*	Negative	Negative	Positive
Mallow	*Malva parviflora*	Negative	Negative	Negative
Strawberry weed	*Modiola caroliniana*	Negative	Negative	Negative
Pig weed	*Portulava oleracea*	Negative	Negative	Negative
Fat hen	*Chenopodium album*	Negative	Negative	Negative
Silverbeet	*Beta vulgaris*	Negative	Negative	Negative
Wild fennel	*Foeniculum vulgare*	Negative	Negative	Negative

Lettuce and field brassicas

Incidence of TuMV varied from farm to farm within both regions surveyed. TuMV in lettuce causes severe stunting of the lettuce and chlorosis of the lower leaves, resulting in unmarketable lettuce. Within the Sydney basin incidence levels TuMV was only found in lettuce on one farm at levels approximately 1.048% (95% confidence limits 0.213-3.046%). It is thought that this is due to the grower planting a newer iceberg variety which had inadvertently been bred away from the TuMV resistance. Most lettuce cultivars planted have broad spectrum resistance to TuMV.

TuMV can be particularly devastating in cabbage, causing necrotic ringspots on the outer leaves. This results in the grower having to manually remove more outer leaves than normal to send to market which results in smaller cabbages with lower weights. Symptom expression of TuMV is temperature dependent as a result surveys were targeted during mid-late winter. TuMV in brassicas was widespread within the Sydney basin and at Bathurst in 2008/2009, with multiple farms having 100% infection (95% confidence limits 19.4%-100%). In the Bathurst region one farm did not have any detectable TuMV within his broccoli crop (incidence level 0% - 95% confidence limits 0-1.2%) despite TuMV being present in wild brassica weeds on the headlands and aphids being present. It is possible that the broccoli being planted was resistant to the local TuMV strains. Weeds which were commonly found with TuMV

were wild turnip (*Brassica tournefortii*), turnip weed (*Rapistrum rugosum*) and wild radish (*Raphanus raphanistrum*). These weeds are well established hosts and growers should be careful to eradicate them where possible as they can serve as virus reservoirs over the summer period when few field brassicas are grown.

Asian vegetables

Analyses of the potyvirus sequences amplified from the coriander revealed two distinct potyviruses: *Apium virus Y* and *Clover yellow vein virus*. It was observed that coriander infected with *Apium virus Y* showed distinct chlorotic blotching, while coriander infected with clover yellow vein virus displayed strong interveinal chlorosis.

Ornamentals

The primers developed by M. Sharman were successful in amplifying INSV while not amplifying TSWV, IYSV or CaCV. The initial survey of the infected property revealed varying levels of infection of INSV between the begonias and spathiphylliums. The infection level in the spathiphyllums was approximately 19% (95% confidence limits 5.611%-14.986%). The infection level in the Begonias was approximately 18.249% (95% confidence limits 11.127%-27.950%). Other plants sampled which were infected with INSV include Lisianthus, Chrysanthemum, Alstroemeria and basil. Of the 11 other ornamental wholesale and retail nurseries surveyed none were positive for INSV, any symptomatic plants collected were infected with TSWV. None of the nearby vegetable producers surveyed were positive for INSV. INSV can be a serious constraint to vegetable production. Recently, in California, it devastated field lettuce production in a manner similar to TSWV.

Glasshouse experimental work

From the mechanical inoculation work fleabane and dock were both shown to be positive to PRSV by ELISA. Only one of the broad bean plants was tested as positive to PRSV. Interestingly all of the fleabane plants tested were shown to be positive to PRSV, despite only a subset being inoculated with PRSV. PCR testing again confirmed PRSV in the fleabane and the Dock. The detection of PRSV in all of the fleabane used in glasshouse work is suggestive that either a large cross contamination occurred during the experiment, the initial seed was contaminated or that there is a cross reaction between the PRSV antisera and some proteins within the fleabane plant. While the third option seems the most likely there is some doubt as many fleabane plants were surveyed within the zucchini survey work and most did not react positively to the PRSV antisera. It maybe a different *Coryza* species was used in the glasshouse experimental work to what was collected and tested consistently from the field. The aphid transmission experiment confirmed that PRSV could be transmitted by cotton aphid fed on PRSV, ZYMV and WMV infected zucchini plants. As previously discussed the fleabane reacted positively to the Agdia antisera for PRSV, WMV and ZYMV. Analyses of the potyvirus sequences amplified from the infected fleabane tissue revealed the presence of PRSV and WMVII.

Acknowledgements

Leanne and Len would like to thank Sylvia Jelinek for collaborating on the majority of the zucchini survey and experimental work. We would also like to acknowledge

Ros Dixon, Aida Ghaliani, Mary Ann Terras, Sue Austin, Tegan Crowe, Jon Collins and Roger Carrus for their technical assistance. This project was linked with other projects funded by RIRDC (DAN233J Integrated management strategies for diseases and pests of Asian vegetables) and HAL (VG03109 and VG03098 Regional extension strategy for managing western flower thrips and tomato spotted wilt virus in the Sydney region).

References:

Constable FE, Nicholas P and Rodoni B (2010) Development and validation of diagnostic protocols for the detection of endemic and exotic pathogens of grapevines. GWRDC Final Report for project Number DPI 05/04.

Moran et al 1983, Plant Disease, 67: 1325-1327.

Rolf and Sokal 1969, Statistical Tables.

Chu FH, Chao CH, Chung MH, Chen CC and Yeh SD (2001) Completion of the genome sequence of watermelon silver mottle virus and utiliaation of degenerate primers for detecting tospoviruses in five serogroups. Phytopathology 91, 361-368.

Colinet D and Kummert J (1993) Identification of a sweet potato feathery mottle virus isolate from China (SPFMV-CH) by the polymerase chain reaction with degenerate primers. Journal of Virological Methods. 45, 149-159.

Appendix 3

Final report Tasmania VG0 7128

Investigators: A/Prof. Calum Wilson[A], Dr. Susan Lambert[B], Dr Alison Dann[A], Dr. Frank Hay[B], and Mr. Peter Cross[C],

[A] Tasmanian Institute of Agricultural Research (TIAR), 13 St. John Ave., New Town, Hobart, Tasmania, Australia 7008.

[B] TIAR, P.O. Box 3523, Burnie, Tasmania, Australia 7320.

[C] Department of Primary Industry, Parks, Water & Environment, , 13 St. John Ave., New Town, Hobart, Tasmania, Australia 7008.

Contact details for project leader:

A/Prof Calum Wilson (Research Leader Vegetable Group)

TIAR, 13 St. John Ave., New Town, Hobart, Tasmania, Australia 7008.

Purpose of project

The aim of this project was to assess virus incidence and prevalence of crops within the Tasmanian processing vegetable industry. The project consisted of a survey of vegetable crops grown in Tasmania including peas, beans carrots, broccoli and cauliflower for the prevalence and incidence of common virus groups and species. These included Potyviruses, Luteoviruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Cauliflower mosaic virus* (CaMV), *Subterranean clover stunt virus* (SCSV) and *Tomato spotted wilt virus* (TSWV).

Media summary

The objective of this study was to provide a benchmark for virus disease status in major Tasmanian processing vegetable crops. A survey for incidence of infection with common virus species or groups was conducted over three consecutive vegetable growing seasons (2008 to 2010 season). The prevalence and incidence of members of the Potyviridae, the Luteoviridae (tested using universal Potyvirus and Luteovirus

antisera which detect most members of these virus families), and specific virus species *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Subterranean clover stunt virus* (SCSV), *Tomato spotted wilt virus* (TSWV) and *Cauliflower mosaic virus (*CMV). In general, relatively low levels of virus infection were detected throughout the survey period suggesting currently that virus diseases have little impact on these processing crops.

Outcomes from this survey will provide valuable information for the Tasmanian processing vegetable industry, highlighting potential key virus pathogens that may require further monitoring, and assist in maintaining high health and quality of Tasmanian processed vegetable crops.

Technical summary

A virus survey was conducted in Tasmania over three consecutive growing seasons (2008, 2009, 2010 seasons) to determine prevalence and incidence of virus within pea (*Pisum sativum* L.), bean (*Phaseolus vulgaris* L.), carrot (*Daucus carota* L.) and brassica crops including broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*) grown in Tasmanian.

Three hundred leaves were randomly sampled from each crop and tested for various viruses or virus groups using enzyme linked immunoassay (ELISA). Legume (pea and bean) crops were tested for Potyviruses, Luteoviruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Subterranean clover stunt virus* (SCSV), and *Tomato spotted wilt virus* (TSWV). Brassica (broccoli and cauliflower) crops were tested for Potyviruses, Luteoviruses, *Cucumber mosaic virus* (CMV) and *Cauliflower mosaic virus (*CMV). Carrot crops were tested for Potyviruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV).

Low (and sporadic) incidence of each virus (or virus group) was detected in pea crops sampled. Only Luteovirus and SCSV infections were found in bean crops (at low frequency and incidence). Carrot crops sampled failed to record any virus infections. Broccoli crops recorded generally low incidence of all four viruses or groups tested for, whilst cabbage crops recorded very low incidence of CaMV only. In the pea and

broccoli crops Luteoviruses were the most prevalent virus group detected. Samples testing positive for Potyvirus and Luteovirus infection were retested using antiserum specific to known virus species. These tests revealed all potyvirus detections (from pea crops in the first year of testing) were due to infection with *Pea seed-borne mosaic virus* (PSbMV). These samples were all sourced from a single imported seed line. Subsequent testing over the next two seasons failed to show evidence of PSbMV which may have been eliminated with destruction of the seed source. Serological retesting of Luteovirus samples revealed 32% of samples tested positive for *Beet western yellows virus* (BWYV), whilst *Bean leafroll virus* and *Soybean dwarf virus* were not detected.

Molecular analyses of Luteovirus isolates collected from these crops revealed most infections were due to *Turnip yellows virus* (a variant of BWYV) with a few isolates from pea belonging to a putative novel luteovirus species with closest homology to *Curcurbit aphid-borne yellows virus.*

Introduction

Vegetable production in Tasmania is a vitally important component of the State's economy. The Tasmanian vegetable production represents 18% of the agricultural value at the farm gate equating to $164M in 2004-05 (Anon. 2009). The majority of vegetables produced in Tasmania in 2004/2005 were grown for the processing sector, (14,000 ha produced for processing and 3,500 ha produced for the fresh market sector; O'Donnell 2006). Vegetable production is predominantly located in the northern cropping regions of Tasmania. In 2004/2005 approximately 90% of the vegetables produced in Tasmania could be attributed to potatoes, onions, carrots, peas, beans and broccoli (O'Donnell 2006). Vegetable crop area and tonnage in Tasmania in 2004/2005 included peas (processed) with 5,580 ha and 32,000 t (shelled), beans with 1,800 ha and 16,000 t, broccoli with 355 ha and 2,700 t, cauliflower with 300 ha and 6,500 t and carrots with 900 ha and 40,000 t (O'Donnell 2006; Anon. 2005). Value of Tasmanian vegetable crops at the farm gate in 2004/05 include peas (process) $13.5M, beans (processed) $6.2 M, broccoli $5.4M , other brassicas $2.7M and carrot $6.2M (O'Donnnell 2006).

Maintenance of pathogens at low incidence in vegetable crops is necessary to ensure sustained quality and crop yields, and to maintain national and international competitiveness of vegetable production within Tasmanian. Surveys provide valuable information of the incidence and prevalence of disease in crops and enable informed decisions to be made regarding mitigation strategies for disease management. With the exception of annual screening of potato crops and specific surveys of carrot crops for *carrot virus Y* and onion crops for *Iris yellow spot virus*, there have been no systematic surveys of vegetable crops in Tasmania for virus infection in recent decades.

There are many (over 50 named) viruses recorded that may infect pea and bean plants (although many of these have not been recorded as field infections). Brassica plants (including cauliflower and broccoli) are known to be susceptible to 10-20 different viruses, and carrots a similar number. In Tasmania records of *Bean yellow mosaic virus* (BYMV), and *Subterranean clover redleaf virus* (syn to Soybean dwarf; SDV) in pea; BYMV, SDV, *Subterranean clover stunt virus, Tobacco yellow dwarf virus*, and *Bean common mosaic virus* in bean; *carrot virus Y*, carrot motley dwarf disease (mixed infection with *carrot red leaf virus* and *carrot mottle virus*) in carrot; *Cauliflower mosaic virus* (CaMV) in cauliflower and broccoli exist (Sampson & Walker; 1982). In addition, *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV), *Tomato spotted wilt virus* (TSWV), and *Beet western yellows virus* (BWYV) are common viruses with broad host ranges that have been recorded in Tasmania and are likely to occur in the target crop species (Johnstone & Duffus, 1984).

The objective of this study was to provide a virus disease status in Tasmanian processing vegetable crops by conducting a virus survey over three consecutive vegetable growing seasons (2008 season to 2010 season). The prevalence and incidence of the Potyvirus and Luteovirus families and *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Subterranean clover stunt virus* (SCSV), *Tomato spotted wilt virus* (TSWV) and *Cauliflower mosaic virus (*CMV) were assessed in pea, bean, carrot, broccoli and cauliflower crops.

Information gained from this survey will provide valuable information for the Tasmanian processing vegetable industry, indicating potential key pathogens that may

require management or monitoring and assist in demonstrating (and maintaining) the high health and quality standard of the Tasmanian vegetable industry.

Materials and methods

Sampling

A total of 134 crops were surveyed over three consecutive years (December 2007 and April 2010).

During the 2008 (December 2007- April 2008) season, 2009 (December 2008 -August 2009) season and 2010 (December 2009-April 2010) season a total of 20, 17 and 19 pea crops were surveyed. In 2008 27 bean crops were surveyed. Both pea and bean crops were tested for prevalence and incidence of six viruses or virus genera (potyvirus, luteovirus, TSWV, SCSV, CMV and AMV; Table 1). In 2010 a total of 19 pea crops were tested for luteovirus and potyvirus infections only. A total of 11 (5 cabbage and 6 broccoli crops), 12 (broccoli crops) and 6 (broccoli crops) brassica crops were tested in 2008, 2009 and 2010 seasons. Tests included luteovirus, potyvirus, CMV and CaMV (Table 1). In 2009 a total of 22 carrot crops were virus tested for potyvirus, AMV and CMV (Table 1).

Three hundred leaf samples were randomly collected from each crop at or after flowering (22 December 2007 – 29 April 2010) from arbitrary locations along irrigation runs and at the field edge.

Within each field, a single leaflet was collected from different plants at or post flowering from arbitrarily chosen locations along transects at field edges and along irrigator and spray rig runs within the crop. This sampling strategy was chosen to minimise movement through a crop that may contribute to mechanical transmission of these viruses. Field hygiene practices were also adopted to reduce the risk of virus transmission between fields, which included the use of disposable boot covers between fields. Leaves were stored at 4ºC for no more than 5 days before virus testing.

Table 1. Number of crops tested for luteovirus, potyvirus, *Alfalfa mosaic virus (*AMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Subterranean clover Stunt Virus* (SCSV), *Cauliflower mosaic virus* (CaMV) of different varieties of Tasmanian vegetable crops collected during the 2008, 2009 and 2010 growing season.

	Number of crops tested						
Crop and season	**Luteovirus**	**Potyvirus**	**AMV**	**CMV**	**TSWV**	**SCSV**	**CaMV**
Pea - Total	**56**	**56**	**37**	**37**	**37**	**37**	**-**
2007/2008	20	20	20	20	20	20	-
2008/2009	17	17	17	17	17	17	-
2009/2010	19	19	-	-	-	-	-
Bean Total (2007/2008 only)	**27**	**27**	**27**	**27**	**27**	**27**	**-**
Carrot Total (2008/2009 only)	**22**	**22**	**22**	**22**	**22**	**22**	**-**
Brassica Total	**29**	**29**	**-**	**23**	**-**	**-**	**29**
2007/2008	11	11	-	11	-	-	11
2008/2009	12	12	-	12	-	-	12
2009/2010	6	6	-	-	-	-	6
Total crops virus tested	**134**	**134**	**86**	**109**	**86**	**86**	**29**

Virus testing

Leaf samples collected from each field were grouped into lots of ten and a subsample (*ca.* 1.0g) was homogenized in a rotary press in 1.0ml of 0.01 M phosphate-buffered saline (pH 7.4) containing polyvinylpyrrolidone (MW 40,000; 20 g/L) bovine serum albumin (2.0 g/L) and Tween 20 (20 ml/L). A universal monoclonal antibody specific for most members of the Potyviridae was obtained from Agdia Inc. (USA) and a universal polyclonal antiserum against members of the Luteoviridae was sourced from DSMZ GmbH (Germany). The presence of virus (TSWV and CaMV) was tested by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adam 1977) using polyclonal antisera (Agdia Inc. Elkart, Indiana, USA) in polystyrene microtitre plates (Nunc) following manufacturers recommended protocols. The presence of CMV and AMV were tested by triple antibody sandwich ELISA using antisera obtained from Agdia Inc. Elkart, Indiana, USA. Antiserum for SCSV was obtained from Peter Cross (Department of Primary Industry, Parks, Water and Environment (DPIPWE), Hobart, Tasmania) and followed DAS ELISA protocol outlined by TASAG ELISA (Peter Cross, pers. Communication, 2008).. A Multiscan photometer (Flow Laboratories, Helsinki, Finland) was used to record the Absorbance (A_{405}) of each well. Where absorbance was greater than the mean absorbance of the negative controls plus three times the standard deviation of buffer only, samples were considered positive (Sutula *et. al.* 1986).

Analysis for virus prevalence

Virus incidence was estimated from grouped samples using the Gibbs and Gower (1960) technique. Virus incidence (%) was estimated from the number of bulked samples identified as infected with singular infections or co-infections of universal luteovirus, universal Potyvirus, TSWV, CMV, AMV, CaMV and SCSV. In this technique:

N is the number of grouped samples

i is the number of leaflets in each group sample

R is the number of grouped samples that give a positive virus test

p is the proportion of infected plants in the crop being tested

*p** is the maximum likelihood estimated of p

p = 1-p is the proportion of uninfected plants in the population and q* = 1-p* is the estimate of q.

The probability that none of i leaflets is infected is q^i and the probability of a positive virus test when i leaflets is tested is 1- q^i. The estimate q^* is given by:

$R/N = 1 - q^{*i}$ Therefore $p^* = 1 - q^* = 1-(1-R/N)^{1/i}$

Retesting of potyvirus and luteovirus samples

Initial screening included antisera that detect a wide range of luteovirus and potyvirus species. Samples testing positive in these tests groups were forwarded to DPIPWE in New Town laboratories for further testing for specific virus species within these families.
These testes included:

Potyviruses (*Pea seed-borne mosaic virus, Bean yellow mosaic virus, Clover yellow vein virus*)

Luteovirus (*Beet western yellows virus, Bean leafroll virus, Subterranean clover red leaf virus*)

The identity of several luteovirus samples were unresolved following serological testing, thus further molecular analysis was conducted on a selection of pea and broccoli samples.
Total RNA was extracted from 100 mg leaf material according to the manufacturer's recommendations in the RNeasy® Plant Mini Kit (Qiagen, Valencia, USA) with the exception of the tissue being homogenised in a FastPrep FP120 instrument for 40 seconds at maximum speed with lysis matrix D (MP Bio, Solon, USA). RNA concentration was estimated using a Picofluor fluorometer (Promega, Sunnyvale, USA) with a Quant-it™ RiboGreen RNA assay kit (Invitrogen, Carlsbad, USA). A working stock of RNA was stored in sterile distilled water at -20°C and an aliquot stored at -80°C.
Reverse transcription was performed using Qiagen LongRange 2 step RT-PCR kit following the manufacturer's protocol, in a 10 µL reaction: 2.5 µL total RNA, x1 RT-PCR buffer, 1mM dNTPs, 1µM oilgo-dT, 0.04U Rnase inhibitor and x1 RT enzyme, made up to volume with sterile distilled DEPC (Diethyl pyrocarbonate) treated water. The reaction was incubated at 42°C for 90 mins and the enzyme deactivated at 85°C for 5 mins.
Polymerase chain reactions using universal *Luteoviridae* primers (Lu1/Lu4, Luteo1F/Luteo1R and C2F1/2R2/3) (Table 2) were performed as follows; 1x HotStarTaq Master Mix (Qiagen), 1 µM of each primer, 1 µL of cDNA template and made up to a total volume of 25 µL with sterile distilled water. The PCR cycling conditions are summarised in Table 2. The ORF0 and ORF3 specific PCRs were performed as follows; 1x HotStarTaq

Master Mix (Qiagen), 1 µM of each primer pair, 1 µL cDNA template and made up to a total volume of 25 µL with sterile distilled water. The PCR cycling conditions are summarised in Table 1. All PCRs were visualised in 1.5% agarose gel electrophoresis with SYBR Safe™ DNA Gel Stain (Invitrogen) in 1X lithium borate buffer. Amplicons that corresponded to the expected size (Table 2) were excised from the gel and purified using a Qiagen QIAEX II Gel Extraction Kit. Template purity was re-checked by agarose gel electrophoresis. Amplicons were sent to AGRF (Australian Genome Research Facility) for sequencing.

The sequences were edited using ChromasPro v1.32 (Technelysium Pty Ltd) and BioEdit v7.0.9 (Hall 1999) and compared to the NCBI GenBank database sequences using BLASTn comparisons. The sequences were aligned with highly matched sequences from the GenBank database using the BioEdit ClustalW alignment application and checked manually. Phylogenetic trees were created with the Neighbor-Joining algorithm with 100 bootstraps and viewed in CLC Bio Sequence Viewer v6.4 (Katrinebjerg, Denmark).

A separate reverse transcription reaction was performed using the above method but with the ORF1 and ORF2 primers (Table 2) designed from a consensus of the ORF1 and ORF3 sequences using Primer3Plus (Untergasser et al. 2007) and used for sequencing by primer walking.

Table 2 Sequences, targets, positions, expected size and cycle conditions for primers used in this study

Name	Sequence 5' to 3'	Target	Position	Expected size (bp)	Reference	PCR conditions
C2F1	TCACKTTCGGGCCGAGT	Partial ORF3		148	Chomič *et al* 2010	1x95°C/15 mins, 15x(94°C/30 secs, 65°C/30 secs-reduce by 1°C per cycle,
C2F2	TCACKTTCGGGCCGTCT			68		72°C/30 secs), 25x(94°C/30 secs, 50°C/30 secs, 72°C/ 30 secs), 1x 72°C/7 mins
C2R1+2	TCMAGYTCGTAAGCGATKG					
C2R3	YTCATGGTAGGCCTTGAG					
TuYVorf0F	ACAAAAGAAACCAGGAGGGAATCCTTA	TuYV ORF0	1	780	Schubert *et al* 1998	1x 95°C/15 mins, 35x 94°C/30 secs, 55°C/30 secs, 72°C/1 min,
TuYVorf0R	TCATACAAACATTTCGGTGTAGAC		781			1x 72°C/10 mins
TuYVCP+	ATGAATACGGTCGTGGGTAGGAG	TuYV ORF3	3483	563	Beuve *et al* 2008	as above 55°C annealing
TuYVCP-	CCAGCTATCGATGAAGAACCATTG		4045			
BWORF0+	ATGCAATTTCTCGCTCACGATAACT	BWYV ORF0	32	750	Hauser *et al* 2000	as above 55°C annealing
BWORF0-	TCATACAAACATTTCGGTGTAGAC		781			
BWYVcpF	CAGTAGCCGGTATTTACTTAGTCTACC	BWYV ORF3	3472	648	this study	as above 56°C annealing
BWYVcpR	GGCACTTCATAGTGATTCTAAAAGAA		4119			
CabYVorf0F	ACAAAAGATACGAGCGGGTGATGCAAA	CabYV ORF0	1	820	this study	as above 58°C annealing
CabYVorf0R	GTCCTTATATTGGACCTGCAAGACCG		821			
CE9	GAATACGGTCGCGGCTAGAAATC	CabYV ORF3	3507	600	Juarez *et al* 2004	as above 58°C annealing
CE10	CTATTTCGGGTTCTGGACCTGGC		4104			
ORF1F	CTCTTCGGTCTACACCGAAATGTTTGTAT	ORF1	751	2810	this study	as above 64°C annealing
ORF2R	CCSAGRGGYTTGGACCACAACCACTSGCTGA	ORF2	3560			

ORF = open reading frame, bp = base pairs

3. Results

3.1 Peas

In 2008 low incidences of luteovirus, potyvirus, AMV, CMV, TSWV and SCSV were detected in pea crops. A total of 12/20 (60%) of pea crops tested in 2008 were infected with luteovirus with a mean incidence of 0.51% (0-2.21%). (Table 3).

Two crops (10%) were coinfected with potyvirus and luteovirus in 2008. In 2008 one crop (5%) was coinfected with AMV, CMV and SCSV, with mean incidences of 0.15% (0-0.34%), 0.05% (0-1.05%) and 0.19% (0-1.05%), respectively. A total of five pea crops were infected with SCSV, with four crops with no co-infections. One pea crop was infected with TSWV with a mean incidence of 0.02% (0-0.34%), with no co-infection reported (Table 3).

In 2009 and 2010 seasons luteovirus was detected at low levels in 60.0% and 58.8% of pea crops respectively with a mean incidence of 1.31% (0-6.70%) and 0.11 (0-3.31%). Potyvirus, AMV, CMV, TSWV and SCSV were not detected in 2009 and 2010 season (Table 3).

3.2 Beans

A total of 2/27 (7.4%) bean crops in 2008 were infected with luteovirus with incidence ranging from 0-0.34% (mean of 0.02%). One bean crop was infected with SCSV with a mean incidence of 0.01% (0-0.34). Potyvirus, AMV, CMV and TSWV were not detected in bean crops in 2008 season. No further virus testing was conducted on bean crops (Table 3).

3.3 Brassica

Luteovirus was detected at low incidence in broccoli crops in 2008 (5/6 crops, 83.3%), 2009 (5/12 crops, 41.6%) and 2010 (5/6 crops, 83.3%) seasons, with mean incidence of 0.68% (0-3.50%), 1.41% (0-6.7%) and 1.21% (0-2.62%), respectively. In 2008 an additional five cauliflower crops were virus tested, one crop was infected with a single infection of CaMV, with a mean incidence of 0.07% (0-0.34%). Luteovirus, potyvirus and CMV were not detected in the remainder of the cauliflower crops tested (Table 3).

A total of 12 broccoli crops were tested in 2009, with luteovirus detected in 5/12 (41.6%) crops with a mean incidence of 1.41% (0-6.7%). Potyvirus was detected as a single infection in one (8.3%) broccoli crop with a mean incidence of 0.03% (0-0.34%). One crop was coinfected with SCSV and luteovirus. CaMV was not detected in broccoli crops in 2009 (Table 3).

In 2010 season luteovirus was reported in 5/6 (83.3%) broccoli crops with a mean incidence of 1.21% (0-2.62%). Potyvirus and CaMV were not detected (Table 3).

3.4 Carrot

Potyvirus, AMV and CMV were not detected in carrot crops in 2008/2009 season. No further virus testing was conducted on carrot crops (Table 3).

3.5 Retesting of Potyvirus and Luteovirus samples

Potyvirus samples:

Retesting positive potyvirus samples from the two pea crops (2008 season) revealed all five samples from one crop were infected with PSbMV (with no detections of BYMV or CYVV), whilst the one sample from the second crop tested negative for all three viruses. No further testing of these samples was done. Both crops were planted with the same imported seed line. Measures were taken to remove this seed line and the crops processed (with no seed retained). Subsequent testing over the next two seasons did not detect further instances of PSbMV.

Luteovirus samples:

ELISA testing in 2008 suggested 8 of 28 luteovirus samples from pea may be infected with BWYV. In 2009 7 of 28 luteovirus samples tested positive for BWYV, with a further two samples giving ambiguous results. No BLRV or SCRLV were detected in either year.

Subsequent molecular analyses provided full or partial sequence data for coat protein (ORF3) and P0 (ORF0) for 31 pea and 23 broccoli samples. Comparison of sequence data with sequences available on the GENBANK data base showed the majority of isolates from pea (22) and all isolates from broccoli showed the greatest homology to *Beet Western yellows virus* (BWYV) or *Turnip yellows virus* (TuYV, a variant of BWYV found in hosts other than beet). The remaining samples for pea (9) showed

closest homology to *Curcurbit aphid-borne yellows virus* (CabYV), but this was not sufficient close to indicate that these isolates belong to this species (10-20% divergent; Table 4, Fig 1). Furthermore, analysis of P0 sequences from these isolates showed closest homology to TuYV, suggesting that these variants may have arisen by recombination events (Table 4).

Figure 1. Phylogenic tree of coat protein sequences of Tasmanian luteovirus isolates (1p-95b) showing two distinct isolates clusters with TuYV and with CabYV group.
p and b after isolates number denotes isolates originated from pea and broccoli respectively

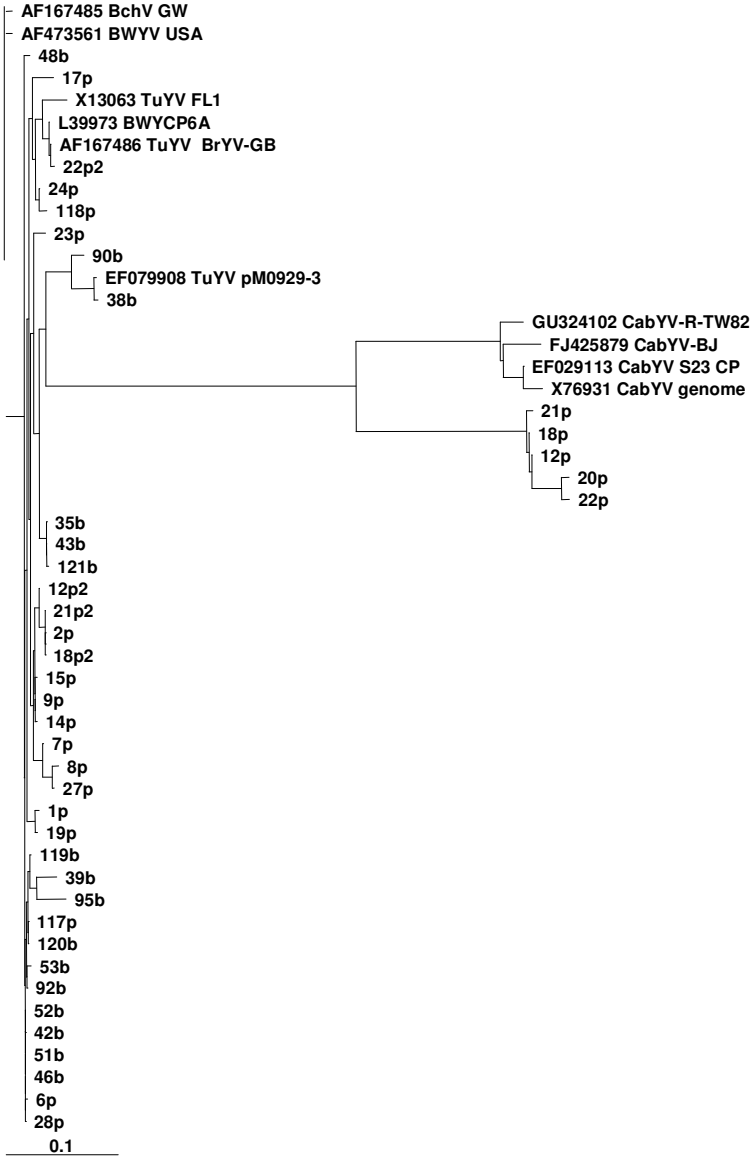

Table 3. Virus incidence of luteovirus, potyvirus, *Alfalfa mosaic virus (*AMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Subterranean clover Stunt Virus* (SCSV), *Cauliflower mosaic virus* (CaMV) of different varieties of Tasmanian vegetable crops collected during the 2007/2008, 2008/2009 and 2009/2010 growing season.

Crop and season	Luteovirus (%)	Potyvirus (%)	AMV (%)	CMV (%)	TSWV (%)	SCSV (%)	CaMV (%)
Pea							
2007/2008	0.51 (0-2.21)	0.15 (0-2.21)	0.02 (0-0.34)	0.05 (0-1.05)	0.02 (0-0.34)	0.19 (0-1.05)	-
2008/2009	1.31 (0-6.70)	0	0	0	0	0	-
2009/2010	0.11 (0-0.34)	0	-	-	-	-	-
Total	**0.61 (0-6.70)**	**0.05 (0-2.21)**	**0.01 (0-0.34)**	**0.03 (0-1.05)**	**0.01 (0-0.34)**	**0.10 (0-1.05)**	**-**
Bean							
Total (2007/2008 only)	**0.02 (0-0.34)**	**0**	**0**	**0**	**0**	**0.01 (0-0.34)**	**-**
Carrot							
Total (2008/2009 only)	**-**	**0**	**0**	**0**	**-**	**-**	**-**
Broccoli							
2007/2008	1.25 (0-0.35)	0	-	0	-	-	0.29 (0-1.42)
2008/2009	1.41(0-6.70)	0.03 (0-0.34)	-	0.03 (0-0.34)	-	-	0
2009/2010	1.21 (0-2.62)	0	-	-	-	-	0
Total	**1.32 (0-6.70)**	**0.01 (0-0.34)**	**-**	**0.02 (0-0.34)**	-	-	**0.07 (0-1.42)**
Cabbage							
2007/2008 (2 crops only)	0	0	-	-	-	-	0.07 (0-0.34)
2008/2009	-	-	-	-	-	-	-
2009/2010	-	-	-	-	-	-	-
Total	**0**	**0**	**-**	**-**	-	-	**0.07 (0-1.42)**

Table 4 Polerovirus specific gene GenBank matches with % match for pea and broccoli samples

Sample type	**Specific gene sequence GenBank Match (%)**			
	Coat protein gene (ORF3)			
	TuYV (EF079908)	BWYV/TuYV (L39973/AF167486)	BWYV/BchV (AF473561/AF167485)	CabYV (EF029113)
P. sativum	-	12 (97-99%)	8 (95-97%)	5 (81-83%)
B. oleracea	2 (96-99%)	13 (94-97%)	2 (97%)	-
	P0 gene (ORF0)			
	TuYV (X13063)	TuYV (AF168608)	BWYV/TuYV(AF168601)	
P. sativum	8 (87-97%)	2 (94-96%)	2 (95-96%)	
B. oleracea	-	-	1 (96%)	
	Partial coat protien gene (partial ORF3)			
	BWYV(AY397615/GU002359)	CabYV(EU636992/GU002335)		
P. sativum	2 (93-99%)	4 (89-90%)		
B. oleracea	7 (96-99%)	-		

GenBank accession number in brackets after Polerovirus match

Discussion

The surveys reported here note very low prevalence and incidence of virus diseases in processed vegetable crops in Tasmania. This was also reflected in a general lack of obvious symptoms associated with virus infections noted during the sampling activities. This suggests the Tasmanian processing industry possesses a high health status with respect to virus diseases. Long rotations with a diversity of crop species and specific seasonal breaks in cropping may account for low virus incidences as potential virus reservoir sources are reduced.

Within the target crops, pea and broccoli had the greatest incidence of virus records, but these were always at levels unlikely to induce economic yields.

No virus infections were detected in carrot crops surveyed in 2008/09. These results reflect similar outcomes found during surveys for CVY in 2000-2002. Within this national study little or no incidence of CVY was detected in Tasmanian carrot crops contrasting dramatically with results from certain other Australian cropping areas within New South Wales, South Australia, Victoria and Western Australia where continuous carrot cropping (with no seasonal break) is often practiced (Latham et al., 2004).

Testing of carrot crops for luteovirus infection (which should detect *Carrot red-leaf virus* incidence of one of the co-infecting viruses responsible for carrot motley dwarf disease) was conducted but not reported due to problems with control material during the carrot surveys making results unreliable. However, no obvious symptoms associated with red leaf or motley dwarf diseases were observed.

In the 2007/2008 season PSbMV was detected in one pea crop with a mean incidence of 2.21%. A second crop tested positive for potyvirus infection, but results from retesting did not confirm PSbMV infection. Both these crops were sourced from the same imported seed line suggestive that the source of infection came with the seed. Low levels of infection despite testing near crop maturity suggested little spread had occurred. The crops were processed and steps taken to eliminate sources of the potentially contaminated seed line. Further PSbMV infections were not detected in pea crops in the 2008/2009 or 2009/2010 surveys.

PSbMV is not listed in the Tasmanian State disease records. This pathogen has been found in most other Australian states in a variety of legume species. Pea seed-borne mosaic virus is a pathogen of potential importance because of its high rate of transmission through seeds. It has posed a threat to the pea seed and processing industry

in USA (Mink et al., 1969; Stevenson & Hagedorn 1971 ; Hampton et al., 1976; Kraft & Hampton 1980). Attempts to eradicate PSbMV in the US had some initial success following its first discovery (Hampton et al., 1976).

The first record of PSbMV in Australia was in quarantine testing of samples from seed imported from Sweden (Munro , 1978). Its natural host range is limited to the Fabaceae family, and it infects pulse crops and pasture legumes. The virus is often symptomless, or shows mild mosaic symptoms on peas however early (substantial) infections may cause considerable yield loses. PSbMV also affects seed quality by causing brown ring patterns and spots on the seeds. Seed transmission of this virus may be as high as 100% in peas (Anon, 2006)

Over the three consecutive years (2008-2010) the most prevelant virus type detected were luteoviruses. Whilst generally present at low incidence most pea (60%) and broccoli crops (63%) had luteovirus infections.

Luteoviruses cause economically important diseases in many crop plants including barley, wheat, potatoes, lettuce, legumes and sugar beets. In Australia luteoviruses have been found in legumes and *Brassica* spp. for many years especially *Beet western yellows virus* (BWYV) (Johnstone et al. 1984; Ashby and Johnstone 1985; Johnstone et al. 1989; Coutts and Jones 2000, 2005). BWYV was first detected in Tasmania in the early 1980s (Duffus and Johnstone 1982) and was subsequently found in a variety of crops including legumes (Johnstone and Duffus 1984) many of which were asymptomatic (Johnstone et al. 1984). Many of the viruses previously named as BWYV (*Beet Western Yellows Virus*) shown not to infect sugarbeet (*Beta vulgaris* L.) are now re-classified as a separate species in the genus *Polerovirus* under the name TuYV (*Turnip Yellows Virus*) (Duffus and Russell 1972; Veidt et al. 1988; Graichen and Rabenstein 1996; Hauser et al. 2000; Hauser et al. 2002). The name *Turnip yellows virus* has now been ratified by the International Committee for the Taxonomy of Viruses (ICTV) (Mayo 2002). In Australia, an isolate from Western Australia (WA-1), analysed by Lemaire and Beuve (pers. comm.) , had a similar nucleotide sequence to that of TuYV isolates from Europe (Coutts et al. 2006). However, insufficient Australian isolates have been sequenced to establish whether BWYV or TuYV predominate, so in previous studies the name BWYV has been used (Coutts and Jones 2000; Coutts et al. 2006; Jones et al. 2007; Maling et al. 2010). No Tasmanian isolates or infected plant samples have been sequenced as far as the authors know at the time of writing this study.

Recent isolates from USA (BWYV-USA) and Europe (Abraham 2005)have been shown to be more related to CabYV than other poleroviruses from Europe (Beuve et al. 2008) and the authors suggest a similar evolution mechanism by intragenus recombination events within the *Polerovirus* genus. (Gibbs and Cooper 1995) suggest two recombination events between BWYV and CabYV occurred. Furthermore, (Pagán and Holmes 2010) calculated very high nucleotide substitution rates in the CP gene for CabYV and clusters of putative recombination breakpoints in BYDV, CabYV, CYDV, ScYLV and TuYV. An alignment of amino acid sequences of the read-through (RT) protein show two distinct regions probably corresponding to separate domains (N-terminal sequences and C-terminal sequences), the N-domain and C-domain. Alignment of the VP (virion protein) amino acid sequences show luteoviruses to have homology with the virion protein shell domain (S-domain) of carmoviruses, sobemoviruses and tombusviruses. The BWYV and CabYV compared by (Gibbs and Cooper 1995) show higher homology to each other in the S-domain and the C-domain amino acid sequences than other luteoviruses. However, the BWYV sequence chosen for this study was BWYV-FL1 now classified as TuYV. In Australia CabYV has not been positively identified in cucurbit crops. However, (Coutts and Jones 2005) found an unidentified luteovirus that reacted with generic monoclonal antibodies to luteoviruses but not with BWYV antibodies infecting cucurbits in Western Australia.

Cucurbit aphid-borne Yellows Virus (CabYV) was first discovered in 1988 as a yellowing disease of melon, cucumber and zucchini in France (Lecoq et al. 1992). Serological analysis, nucleic acid hybridisation and host range studies indicated the virus was related to, but distinct from, BWYV. Lecoq et al. (1992) found their CabYV isolates could be detected with coat protein ORF (open reading frame) RNA probes designed on the isolate BWYV-FL1 which has now been reclassified as TuYV (Mayo 2002) but none of the other ORF RNA probes. Host ranges did overlap, with CabYV and BWYV-FL1 infecting beet (*Beta vulgaris*), lettuce (*Lactuca sativa*) and some Cruciferae genera and there were co-infections. However, BWYV-FL1 did not infect cucurbits and CabYV did not infect legumes or Solanaceae genera. CabYV was also found naturally occurring in weeds in a field where zucchini was the previous crop. CabYV has since been found in many cucurbit crops around the Mediterranean: Italy (Tomassoli and Meneghini 2007), Turkey (Yardimci and Özgönen 2007), Iran (Bananej et al. 2006), Spain (Juárez et al. 2004), Lebanon (Abou-Jawdah et al. 1997), as well as in Asia (Xiang et al. 2008) and the USA (Lemaire et al. 1993).

Poleroviruses are transmitted obligatorily by aphid vectors in a persistent circulative, non-propagative manner and are confined to the phloem. BWYV (TuYV) and CabYV are both principally transmitted by *Myzus persicae* and *Aphis gossypii*, both of which are prevalent in Tasmania. BWYV (TuYV) is internalised at the posterior midgut while CabYV is unique in that it is internalised in both the posterior midgut and hindgut (Reinbold et al. 2001; Gray and Gildow 2003; Reinbold et al. 2003). Because of the difficulty in identifying different Poleroviruses by host ranges, symptoms and ELISA, sequencing the cDNA has become popular, however, coat protein gene sequences, traditional used to identify different luteoviruses, has also been insufficient as many of the new taxa have similar sequences (de Miranda et al. 1995; Schubert et al. 1998; Hauser et al. 2000; Stevens et al. 2005). (Chomič et al. 2010) have designed a suite of generic primers for detecting around 16 Luteovirids.

Acknowledgments

The authors would like to gratefully acknowledge the financial support from the Commonwealth Government through Horticulture Australia Ltd. The authors would also like to thank Mr. Craig Palmer and Mr. Chris Arvia from TIAR for assistance with sampling. In addition we also appreciate assistance with additional virus testing by Mr. Peter Cross and staff (Department of Primary Industry, Parks, Water and Environment, Hobart, Tasmania).

References

Abou-Jawdah, Y., H. Sobh, and A. Fayyad. 1997. First report of Cucurbit aphid-borne yellows Luteovirus in Lebanon. *Plant Disease* 81 (11):1331.

Anon. 2005. Tasmanian vegetable industry situation paper. Department of Primary Industries, Water and Environment, and Department of Economic Development, published. Hobart, Tasmania 3 August 2005 online [http://www.stors.tas.gov.au/au-7-0037-00125].

Anon. 2006. Temperate Pulse Viruses: Pea Seed-borne Mosaic Virus (PSbMV). AgNote (AG1267). Department of Primary Industries, Victoria.

Anon. 2009. Tasmanian Vegetable Industry Scorecard: 2004-05. Food and Agriculture. Department of Primary Industries, Water and Environment. 5 August 2009. Online [http://www.dpiw.tas.gov.au/internnsf/WebPages/CART-76G6G9?open].

Abraham, A. D. 2005. Characterization and genome organization of new luteoviruses and nanoviruses infecting cool season food legumes. PhD Thesis, Faculty of Agricultural Sciences, Georg August University, Göttingen, Germany.

Ashby, J. W., and G. R. Johnstone. 1985. Legume luteovirus taxonomy and current research. *Australasian Plant Pathology* 14 (1):2-7.

Bananej, K., C. Desbiez, C. Wipf-Scheibel, I. Vahdat, A. Kheyr-Pour, A. Ahoonmanesh, and H. Lecoq. 2006. First report of cucurbit aphid-borne yellows virus in Iran causing yellows on four cucurbit crops. In *SeedQuest*: International Society for infectious Diseases.

Beuve, M., M. Stevens, H.-Y. Liu, W. M. Wintermantel, S. Hauser, and O. Lemaire. 2008. Biological and molecular characterization of an American sugar beet-infecting *Beet western yellows virus* isolate. *Plant Disease* 92 (1):51-60.

Chomič, A., M. N. Pearson, G. R. G. Clover, K. Farreyrol, D. Saul, J. G. Hampton, and K. F. Armstrong. 2010. A generic RT-PCR assay for the detection of Luteoviridae. *Plant Pathology* 59 (3):429-442.

Clark, M.F., and Adams, A.N. (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34,** 475-483.

Coutts, B. A., and R. A. C. Jones. 2000. Viruses infecting canola (*Brassica napus*) in south-west Australia: incidnece, distribution, spread and infection reservoir in wild radish (*Raphanus raphinistrum*). *Australian Journal of Agricultural Research* 51:925-936.

Coutts, B. A., and R. A. C. Jones. 2005. Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. *Australian Journal of Agricultural Research* 56:847-858.

Coutts, B. A., J. R. Hawkes, and R. A. C. Jones. 2006. Occurrence of *beet western yellows virus* and its aphid vectors in over-summering broad-leafed weeds and volunteer crop plants in the granite belt of south-western Australia. *Australian Journal of Agricultural Research* 57:975-982.

de Miranda, J. R., M. Stevens, E. de Bruyne, H. G. Smith, C. Bird, and R. Hull. 1995. Beet luteovirus coat protein sequence variation. *Annals of Applied Biology* 127 (1):113-124.

Duffus, J. E., and G. E. Russell. 1972. Serological relationship between Beet western Yellows and Turnip yellows viruses. *Phytopathology* 62:1274-1277.

Duffus, J. E., and G. R. Johnstone. 1982. The probable long association of beet western yellows virus with the potato leaf roll syndrome in Tasmania. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22:353-356.

Gibbs, A.J., and Gower, J.C. (1960) The use of multiple-transfer method in plant virus transmission studies - some statistical points arising in the analysis of results. *Annals of Applied Biology* **48,** 75-83Gibbs, M. J., and J. I. Cooper. 1995. A recombinational event in the history of Luteoviruses probably induced by base-pairing between the genomes of two distinct viruses. *Virology* 206 (2):1129-1132.

Graichen, K., and F. Rabenstein. 1996. European isolates of beet western yellows virus (BWYV) from oilseed rape (Brassica napus L. ssp. napus) are non-pathogenic on sugar beet (Beta vulgaris L. altissima) but represent isolates of turnip yellows virus (TuYV). *Journal of Plant Diseases and Protection (Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz)* 103 (3):233-245.

Gray, S. M., and F. E. Gildow. 2003. Luteovirus-aphid interactions. *Annual Review of Phytopathology* 41:539-566.

Hampton R. 0, Mink G. I., Hamilton R. L, Kraft J. M., 1976. Occurrence of pea seed-borne mosaic virus in North American pea breeding lines, and procedures for its elimination. *Plant Dis. Rep*.,60, 455-459.

Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Research Symposium Series* 41:95-98.

Hauser, S., M. Stevens, C. Mougel, H. G. Smith, C. Fritsch, E. Herrbach, and O. Lemaire. 2000. Biological, serological and molecular variability suggest three distinct polerovirus species infecting beet or rape. *Phytopathology* 90 (5):460-466.

Hauser, S., M. Stevens, M. Beuve, and O. Lemaire. 2002. Biological properties and molecular characterization of beet chlorosis virus (BChV). *Archives of Virology* 147:745-762.

Johnstone, G. R., J. W. Ashby, A. J. Gibbs, J. E. Duffus, G. Thottapilly, and J. D. Fletcher. 1984. The host ranges, classification and identification of eight persistent aphid-transmitted viruses causing disease in legumes. *Netherlands Journal of Plant Pathology* 90 (6):225-245.

Johnstone, G. R., and J. E. Duffus. 1984. Some luteovirus disease in Tasmania caused by Beet western yellows and subterranean clover red leaf viruses. *Australian Journal of Agricultural Research* 35:821-830.

Johnstone, G. R., J. E. Duffus, and P. L. Guy. 1989. New records on the occurrence of beet western yellows virus in Australia, New Zealnad and Mexico. *Australian Journal of Agricultural Research* 40:353-358.

industry: Tasmania. Department of Primary Industries, Water and Environment, Tasmania.

Pagán, I., and E. C. Holmes. 2010. Long-term evolution of the *Luteoviridae:* Time scale and mode of virus speciation. *Journal of Virology* 84 (12):6177-6187.

Reinbold, C., F. E. Gildow, E. Herrbach, V. Ziegler-Graff, M. C. Goncalves, J. F. J. M. van den Heuvel, and V. Brault. 2001. Studies on the role of the minor capsid protein in transport of Beet western yellows virus through Myzus persicae. *Journal of General Virology* 82 (8):1995-2007.

Reinbold, C., E. Herrbach, and V. Brault. 2003. Posterior midgut and hindgut are both sites of acquisition of Cucurbit aphid-borne yellows virus in Myzus persicae and Aphis gossypii. *Journal of General Virology* 84 (12):3473-3484.

Sampson PJ & Walker J 1982. An annotated list of plant diseases in Tasmania. Department of Agriculture, Tasmania.

Schubert, J., F. Rabenstein, K. Graichen, and K. Richter. 1998. Comparison of the 5'-end nucleotide sequences of luteoviruses from oilseed rape and sugarbeet.

Appendix 4

Survey of viruses infecting lettuce crops in Eastern Australia states

Cherie Gambley

Abstract

Lettuce big vein virus and Mirafori lettuce virus were commonly found in lettuce crops surveyed in eastern Australia. Lettuce big vein disease (LBVD) was the most commonly found disease in these surveys but was not always apparent irrespective of the presence of LBVV and MiLV. Sequence of Australian isolates of LBVV and MiLV were obtained are identical to those reported from overseas. Preliminary results support the suggestion that MiLV is the causal agent of LBVD, however, further studies on this are required to confirm.

Introduction

Lettuce is an important crop in Australia, with 165,000 tonnes produced annually. Most of this production is concentrated in the eastern Australian states of QLD and NSW with next largest growing area in WA (ABARE 2009). Many viruses are known to affect lettuce in Australia, including *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Lettuce mosaic virus*, *Turnip mosaic virus* and *Lettuce necrotic yellows virus*. In addition Lettuce big vein disease, a disease suspected to have a viral etiology, is present in most growing areas. Lettuce big vein virus (LBVV) and Mirafori lettuce virus (MiLV)

are commonly found in lettuce overseas and thought to have a role in lettuce big vein disease. This study was conducted to ascertain the most important virus diseases affecting Australian lettuce.

Materials and methods

Virus surveys and isolates

Lettuce crops located in the Lockyer valley were surveyed in late winter, early spring 2008 whereas those in the Stanthorpe region were done in summer 2009. A total of five lettuce crops were surveyed. Two lettuce crops in winter near Gatton, 200 random samples collected from each and selected symptomatic samples collected, mostly LBVD no other viral disease suspected. One lettuce crop on the Gatton research station surveyed early spring, 300 random samples collected plus symptomatic samples. Another commercial crop surveyed early spring in Gatton district, LBVD obvious, no other viral disease suspected but symptomatic and 300 random samples collected. The Stanthorpe crop showed no obvious viral diseases, 300 random samples collected.

Virus isolates were also collected during farm visits and/or submitted by growers. This includes samples from both field and hydroponic grown crops.

Molecular indexing

Total nucleic acid extractions (TNAEs) were prepared from 0.03-0.06 g of leaf laminar tissue using a QIAGEN Biosprint 15 DNA Plant Kit, according to the manufacturer's instructions and nucleic acids eluted from the beads in 200 µl of water. Random samples were bulked in lots of ten for TNAEs but symptomatic samples were extracted individually. Samples were indexed by RT-PCR for TSWV, MiLV, LBVV and CMV. Primers used for virus detection are shown in Table 1.

Table 1. Primers used for diagnostic indexing.

Primer	Sequence	Target	Reference
OP1	GGAAGRTATTTYCATAGRGC	RNA1, Ophioviruses	[10]
OP4	GGGTTRTCYTTDATGTAAAATTGACA	RNA1, Ophioviruses	Gambley, unpublished
LB58	GGAAAGACTGCCGGGAAAGAA	LBVV	[10]
LB557	GCGCACTTGTTGTTTGTCGTG	LBVV	[10]
TSWV-NPF	TTAAGCAAGTTCTGTGAGTT	TSWV, NP gene, S-RNA	
TSWV-NPR	ATGTCTAAGGTTAAGCTCACTA	TSWV, NP gene, S-RNA	
CMV5'	TATGATAAGAAGCTTGTTTCGCGCA	CMV	Bariana, et al., 1994
CMV3'	TTTTAGCCGTAAGCTGGATGGACAACCC	CMV	Bariana, et al., 1994

For RT-PCR 2 µl of TNAE was incubated with 500 ng of random hexamers, 100 U of superscript III reverse transcriptase (Invitrogen), 5 × first strand reaction buffer (Invitrogen), 10 U of RNAguard (Invitrogen), 10 mM DTT and 500 µM dNTPs, in a total volume of 20 µl. The reactions were incubated at 50ºC for 1 h then heated at 70ºC for 15 min.

To index for LBVV, each PCR used 2.5 pmoles each of forward and reverse primer, respectively, 2.0 µl of cDNA as template, 10 × PCR buffer (Invitrogen), 1.5 U of *Taq* DNA polymerase (Invitrogen), 1.75 mM $MgCl_2$ and 200 µM of each dNTP, in a total volume of 25 µl. Thermocycling parameters were one cycle at 94ºC for 1 min then 35 cycles at 94ºC for 20 sec, 55ºC for 20 sec and 72ºC for 30 sec and finally one cycle at 72ºC for 5 min.

To index for CMV and TSWV, cDNA reactions were bulked by five and 2.0 µl of the bulked cDNA mix used in each PCR. The remaining PCR reagents and cycling parameters were as above for LBVV.

To index for MiLV, each PCR used 4.0 µl of cDNA as template, 5 pmoles each of forward and reverse primer, 10 × PCR buffer (Invitrogen), 2.5 U of *Taq* DNA polymerase (Invitrogen), 1.75 mM $MgCl_2$ and 200 µM of each dNTP, in a total volume of 50 µl. Thermocycling parameters were one cycle at 94ºC for 1 min then 35 cycles at 94ºC for 20 sec, 50ºC for 20 sec and 72ºC for 30 sec and finally one cycle at 72ºC for 5 min.

As the primers used to detect MiLV are group-specific degenerate primers designed to detect Ophioviruses, further analyses of the PCR amplicons were required to confirm the identity of the virus as MiLV. Therefore PCR amplicons were either sequenced or tested by restriction fragment polymorphisms (RFLP) analysis using the two endonuclease enzymes DraI and EcoRV. To do enzyme digests, 10 µl of each PCR was incubated with 5 U of enzyme for 1 h at 37ºC using the corresponding 10 × reaction buffer in a total volume of 15 µl. Digested DNA fragments were evaluated by electrophoresis using a 2% agarose gel, stained with ethidium bromide and visualised by UV light. The predicted RFLPs for MiLV and two related ophioviruses, Lettuce ring necrosis virus (LRNV) and Ranunuclus white mottle virus (RWMV), generated with the two enzymes are shown in Table 2.

Table 2. Predicted RFLPs for ophioviruses digested with two separate restriction enzymes. DNA fragment sizes are shown in base pairs and 926 bp represents an uncut fragment.

Virus	DraI	EcoRV
MiLV	751 + 175	88 + 467 + 371
LRNV	649 + 277	926
RWMV	926	405 + 521

Soil transmission

A total of seven inoculum soil sources were collected, air-dried for 48 h and tested for transmission of LBVV and MiLV. All seven soils were collected from around the roots lettuce plants infected with both MiLV and LBVV. Each 6 inch pot contained approximately 30-60 g of the air-dried soil inoculum mixed with sufficient sterile potting mixture to fill the pot, then ten lettuce seeds were

added. For each inoculum source a pot each of iceberg and cos lettuce varieties were tested. The plants were germinated and grown in a temperature controlled growth cabinet maintained at 20-22°C, monitored for symptom development and then indexed by RT-PCR for virus presence. The four control cos and iceberg plants were bulked as two separate samples and indexed by RT-PCR, test plants were indexed as individual samples.

Results

Surveys

LBVV and MiLV were detected five crops surveyed whereas neither CMV nor TSWV were detected from any of the samples. No symptoms of potyvirus or LNYV infections were observed during the surveys so samples were not indexed for these viruses. A selection of individual symptomatic plants from two properties in Gatton were collected and indexed separately.

At three sites LBVV was more commonly found in randomly collected samples than MiLV and at the remaining two sites the incidence of both viruses was very similar (Table 3). The incidence of the two viruses in individually selected symptomatic plants was similar to that observed from the random samples (Table 4). There was no clear association of either virus alone or together with disease symptoms.

Variability in the incidence of disease symptoms was also observed between survey sites. Given the viruses are detected at all sites, it is probable that environmental conditions were the key factor in the presence or absence of LBVD symptoms.

Table 3. Summary of virus incidence in randomly collected survey samples.

Property	MiLV	LBVV	total bulks	survey date	Location	Incidence of LBVD symptoms
PMG	6	18	20	6/08/2008	Gatton	high
PMD	18	19	20	6/08/2008	Gatton	high
PGRS	6	16	30	9/09/2008	Gatton	low
PRF	6	22	30	17/10/2008	Gatton	
PRT	18	16	30	19/02/2009	Stanthorpe	very low

Table 4. Summary of virus incidence in individual symptomatic survey samples.

Property	MiLV	LBVV	NVD	Dual	total
PMG	3	8	2	3	10
PMD	23	21	1	22	24

Soil transmission

LBVV and MiLV were transmitted from several inoculum sources either as single or dual infections (Tables 4 and 5). All plants were grown under the same environmental conditions, however, differences were observed in the incidence of symptoms, irrespective of virus infection. It is probable that the variation in there was differences in the concentration of viruliferous olpidium within the soil used as inoculum. No virus was detected in the control plants.

Table 5. Summary of virus transmission from infested soil to cos and iceberg lettuce.

Inoculum	Cos lettuce			Iceberg lettuce		
	symptoms	LBVV	MiLV	symptoms	LBVV	MiLV
2351	0/9	9	0	0/4	4	0
Wer001	0/2	nt	nt	2/10	10	4
Wer002	4/4	o	4	2/3	1	3
Wer003	5/9	8	4	2/8	7	1
Wer004	0/7	7	0	3/5	2	2
control	0/4	0	0	0/4	0	0

Summary of virus incidence vs symptoms

Table 6. Summary of virus incidence in relation to Lettuce big vein disease symptoms from soil transmission tests.

Symptoms	Virus	Percent	Proportion
+	-	1.7	1/59
+	+	28.8	17/59
-	+	67.8	40/59
-	-	1.7	1/59
+	LBVV	16.9	10/59
+	MiLV	23.7	14/59
-	LBVV	64.4	38/59
-	MiLV	6.8	4/59

Approximately 30% of plants showed signs of LBVD and virus was detected in all but one of these plants. Interestingly, there was a high incidence (67.8%) of virus infection without disease symptoms. LBVV was detected in most of these asymptomatic infections. In most MiLV infections LBVD symptoms developed, only 4 of the 18 MiLV-infected plants were asymptomatic at the time of testing. It is possible these plants were infected later than those with symptoms and if grown longer would also become diseased.

Sequence characterisation

LBVV sequences were obtained from several survey samples and were almost identical to previously published sequences of the virus. By contrast, when the PCR amplicons generated using the OP1/OP4 primers from several samples were digested with restriction enzymes, two unique RFLPs were detected. Sequencing of these amplicons revealed there are two distinct sequence variants of MiLV in Australia which are approximately 91% identical to each other. Several isolates have MiLV sequences which are 98-100% identical to the published isolate LS301-O (GenBank

Discussion

Lettuce big vein associated virus (LBBV) and Mirafiori lettuce virus (MiLV; synonym Mirafiori lettuce big-vein virus, MLBVV) are both transmitted by the soil-borne fungus *Olpidium virulentus* [8] and have been reported to have a role in the development of lettuce big vein disease (LBVD). However, further research has clarified that infection by MiLV alone is responsible for LBVD outbreaks [2, 4-6, 9]. The etiology of LBVD in Australia is largely unknown. Research from production areas in Western Australia supports these findings whereas the disease in eastern Australian production areas had not been investigated.

Tobacco stunt virus (TStV) is thought to be a strain of LBBV [7], thus making LBBV the only species within the genus *Varciosavirus.* MiLV is one of several species of the *Ophiovirus* genus. Viruses within this genus have genomes of 11.3-12.5 kb consisting of three to four ssRNA segments, with RNA 1 at 7.5-8.2 kb in size, representing the majority of the genome ([1]. RNA molecules of both

polarities have been reported and RNA-1 is thought to be negative sense and to encode the RdRp plus another small ORF. RNA-3 is also negative sense and encodes the cp. The amino acid coat protein sequences of CPsV, MiLV and LRNV are 31-52% identical, whereas isolates from the same species almost 100% identical. The homology between the N-terminus of the coat protein of MiLV and TMMMV is about 80%. In a study of 17 MiLV isolates collected from eight different countries the genetic diversity of the complete viral coat protein nucleotide sequences ranged from 0.2% to 12% and the phylogenetic analysis revealed two distinct subgroups which were not correlated with symptom development on lettuce or the geographic origin of the virus isolate [3].

In Australia we have shown that LBVV and MiLV are present in field and hydroponic lettuce grown in the eastern states. Circumstantial evidence from transmission studies and surveys indicate MiLV is more important in disease development than LBVV but further research is needed to confirm this. Further study on alternative weed hosts and longevity of viruliferous Olpidum in hydroponic systems and field soil is needed to begin development of management strategies for control of the disease. The yield loss and affect on shelf life of lettuce affected by the disease or even infected by one or both viruses is largely unknown and warrants investigation.

References:

1. Fauquet C, Mayo M, Maniloff J, Desselberger U, Ball L (2005) Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA
2. Lot H, Campbell RN, Souche S, Milne RG, Roggero P (2002) Transmission by Olpidium brassicae of Mirafiori lettuce virus and Lettuce big-vein virus, and Their Roles in Lettuce Big-Vein Etiology. Phytopathology 92: 288-293
3. Navarro JA, Torok VA, Vetten HJ, Pallas V (2005) Genetic variability in the coat protein genes of lettuce big-vein associated virus and Mirafiori lettuce big-vein virus. Archives of Virology 150: 681-694
4. Plesko IM, Marn MV, Zerjav M (2009) Identification of Lettuce big-vein associated virus and Mirafiori lettuce big-vein virus Associated with Lettuce Big-Vein Disease in Slovenia. Plant Disease 93: 549-549
5. Roggero P, Ciuffo M, Vaira AM, Accotto GP, Masenga V, Milne RG (2000) An Ophiovirus isolated from lettuce with big-vein symptoms. Archives of Virology 145: 2629-2642

6. Roggero P, Lot H, Souche S, Lenzi R, Milne RG (2003) Occurrence of Mirafiori lettuce virus and Lettuce big-vein virus in relation to development of big-vein symptoms in lettuce crops. European Journal of Plant Pathology 109: 261-267

7. Sasaya T, Ishikawa K, Kuwata S, Koganezawa H (2005) Molecular analysis of coat protein coding region of tobacco stunt virus shows that it is a strain of Lettuce big-vein virus in the genus Varicosavirus. Archives of Virology 150: 1013-1021

8. Sasaya T, Koganezawa H (2006) Molecular analysis and virus transmission tests place Olpidium virulentus, a vector of Mirafiori lettuce big-vein virus and tobacco stunt virus, as a distinct species rather than a strain of Olpidium brassicae. Journal of General Plant Pathology 72: 20-25

9. Sasaya T, Fujii H, Ishikawa K, Koganezawa H (2008) Further evidence of *Mirafiori lettuce big-vein virus* but not of *Lettuce big-vein associated virus* with big-vein disease in lettuce. Phytopathology 98: 464-468

10. Vaira AM, Accotto GP, Costantini A, Milne RG (2003) The partial sequence of RNA 1 of the ophiovirus Ranunculus white mottle virus indicates its relationship to rhabdoviruses and provides candidate primers for an ophiovirus-specific RT-PCR test. Archives of Virology 148: 1037-1050

Appendix 5

VG07128 – Integrated Viral disease Management in Vegetable crops

1/12/2007 – 23/12/2010

Victorian Report
– compiled by Brendan Rodoni Senior research Scientist – Department of Primary Industries Victoria

Introduction:
The Australian vegetable industry was valued at around $1.5 billion dollars in 2007/08 and Victoria comprises approximately 25% of this industry and is currently the biggest contributor to this industry on a state by state basis (The National Plant health status Report 2008/09). Whilst only representing a small portion of production, fresh vegetables are a major export industry. In 2007/08 Australia's total vegetable exports was valued at $234 million.

Vegetable crops in Victoria which are frequently severely affected by virus diseases include vegetable cucurbits and lettuce. Losses occur both from direct loss of production and a reduction in the length of the growing season as disease levels and vector populations rapidly increase.

The aim of the project is to enhance the capacity of the vegetable industry to implement integrated virus disease management programs and reduce the economic impact of these in a range of vegetable crops. In year 1 Cucurbits and possible alternative hosts of cucurbit viruses will be surveyed in Swan Hill. Review virus outbreak reports and

investigate epidemics. In year 2 the virus disease surveys will continue in **c**ucurbits as well as lettuce. New viruses and new natural hosts of known viruses will almost certainly be found during the surveys. These viruses will be identified and characterised to a level that allows publication in a peer reviewed journal.

Where possible project staff will arrange and facilitate workshops on integrated virus disease management, targeting consultants and industry representatives. In the third year the district surveys will be completed and a draft version of a review paper on the impact of climate change on virus diseases in vegetables will be completed.

CUCURBITS - Victoria

Introduction:

Cucurbits belong to the family Cucurbitaceae and comprise frost sensitive, tendril-bearing vines growing predominantly in subtropical and tropical regions (Persley et al., 2010). The most important cultivated species in Australia are cucumber, rockmelon, watermelon, pumpkin squash and zucchini with zucchini, pumpkins and cucumbers being the most widely grown in Victoria.

Cucurbits are susceptible to a wide range of fungal bacterial and viral pathogens and parasitic nematodes. However a detailed survey of the viruses of cucurbits has not been conducted. We report here the findings of surveys conducted between 2008 – 2010 from Murray, Gippsland and Mornington Peninsula vegetable growing districts in Victoria. Surveys of cucurbit crops were conducted over several years in Victoria to accommodate the various cropping locations, variations in virus and vector activity between years and distribution of potential alternative weed hosts.

Methods

Survey methods

The method used for the cucurbit survey in Victoria was in line with the sampling and testing range used for similar surveys in Queensland. Accurate data on crop locations, disease incidence and symptoms, cultivars collected and crop age was collected when ever possible. Sampling consisted of a 200 random leaf samples /crop which are tested for a range of viruses in groups of 10 using ELISA. Plantings of approximately the same age on one farm can be one crop. Symptomatic plants representing the range of symptom types seen can be collected as individual additional samples.

Visual estimates of virus symptoms were made and samples collected for further analysis. At each property a 200 leaf sample was collected by ELISA for *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Papaya ringspot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV). Samples representing "plants of interest" were also collected and screened for the same viruses using ELISA. Agdia ELISA kits were used for each ELISA test unless otherwise stated. In some instances additional PCR tests were conducted on some plants.

Results and Discussion:

2008 survey – Swan Hill – Mildura Region:

In March 2008 10 properties growing cucurbits along the Murray on the Victorian/NSW border between Swan Hill and Mildura were surveyed for viruses. Table 1 summarises

the crops, viruses detected and associated incidence of disease based on visual symptoms. For a complete set of survey results please refer to Appendix 1.

CMV, SqMV, PRSV and ZYMV were not detected in any samples tested at any of the properties tested in the Swan Hill and Mildura districts in March 2008.

WMV was detected in 4/20 of the pooled samples of zucchini at property 1 and equates to an estimate of 2.15% levels of WMV infected plants in the Zucchini crop. For property 2 WMV was detected in 20/20 of the pooled zucchini samples and this equates to an estimate of 31.75% levels of WMV incidence in the crop. It is likely that the true incidence is higher. At property 3 WMV was detected in 7/20 of the pooled samples and equates to an estimated level of 4.00% WMV in the Zucchini crop. For both properties 4 and 5 WMV was detected in 20/20 of the pooled samples and it is likely that the true levels of infection are higher than the estimated 31.75%.
At property 6 WMV was detected in only 1/20 of the pooled samples and equates to 0.5% infection levels of WMV in the crop.

At properties 7 – 9 none of the 5 viruses tested for using ELISA were detected.

Table 1: Cucurbit crops surveyed, symptoms observed and viruses detected using ELISA in March 2008.

Property	Crop/s	Description	Viruses detected
1	Squash (sunburst)	Severe leaf and fruit mosaic (<1%)	WMV (4/20 +ve)
	Zucchini (Congo)	Severe leaf and fruit mosaic (<1%)	
2	Zucchini (Congo)	Severe to very severe leaf mosaic (>95%)	WMV (20/20 +ve)
3	Zucchini (Congo – mature crop)	Mild to moderate mosaic (5% incidence)	WMV (7/20 +ve)
	Zucchini (Congo – young crop)	Healthy	
4	Zucchini (Congo – fruiting)	Moderate to severe mosaic (> 95%) 200 leaf collected from the Zucchinis	WMV (20/20 +ve)
	Pumpkins (senescing) Squash (Sunburst (fruiting)	Mild mosaic symptoms	
5	Zucchini (Congo – fruiting)	Mild mosaic symptoms (25% incidence)	WMV (20/20 +ve)
6	Zucchini (Congo – fruiting)	Mild mosaic (,1% incidence)	WMV (1/20 +ve)
7	Watermelon (senescing)	Healthy (200 leaves for testing collected from watermelons)	No virus detected.
	Rockmelon (fruiting)	Healthy	
8	Honeydews/ Rockmelons (fruiting)	Healthy	No virus detected.
9	Zucchini (Caledia – fruiting)	Healthy	No virus detected

At each property visited in the 2008 survey individual samples were collected and screened for virus using ELISA and PCR where appropriate. Table 2 summarises the results from these samples.

Of the individual plants that were sampled at Property 1 (TF021-TF027 – Appendix 1)) there were some interesting results. WMV was detected in a squash sample displaying the "Aussie Gold" symptom (TF023) while no virus was detected in another sample (TF021) showing the same symptom. This result suggests that an additional factor, possibly a virus, is associated with the "Aussie Gold" symptom. WMV was detected in a zucchini plant showing severe leaf distortion (TF024), a symptom more typical of those associated with PRSV. Zucchini plants expressing milder mosaic and leaf distortion symptoms were also infected with WMV. This observation also suggests that an additional factor is involved in symptom expression in cucurbits in the Swan Hill area.

At property 2 a paddymelon was found to be infected with WMV (MB021) and this highlights the role this weed can play as an alternate host of WMV and a source of inoculum. Only WMV was detected in the zucchini samples, even though there were

Table 2:

Property	Sample ID	Sample description	Viruses detected
1		Aussie Gold squash, symptomatic, fruit in same bag but	-ve
	TF021	from different plant same leaf symptoms;	
	TF022	single zucchini sample	-ve
	TF023	Aussie gold squash	WMV +ve
	TF024	PRSV-like symptoms in zucchini	WMV +ve
	TF025	erect, yellow symptom in zucchini	-ve
	TF026	odd, possible nutritional	-ve
	TF027	individual plant sample of squash;	-ve
2	MB021	paddy melon possible symptoms	WMV +ve
	MB022	mosaic,	WMV +ve
	MB023	golden mosaic symptom,	WMV +ve
3	SH2021	symptomatic plant from the oldest zucchini bays	WMV +ve
	SH2022	symptomatic plant from the oldest zucchini bays	WMV +ve
	SH2023	symptomatic plant from the oldest zucchini bays	WMV +ve
	SH2024	symptomatic plant from the oldest zucchini bays	WMV +ve
		paddy melon random sample of bulk ten plants collected	WMV +ve
	SH2025	from younger zucchinis	
	SH2026	symptomatic plant from the oldest zucchini bays	WMV +ve
	SH2027	symptomatic plant from the oldest zucchini bays	WMV +ve
		paddy melon random sample of plants collected from	WMV +ve
	SH2028	headland adjacent to oldest zucchini planting	
4		PRSV-like symptom, severe and typical for the crop,	WMV +ve
	SH021	light-dark green mosaic, h/r no shoe-string symptom	
		mosaic symptom on squash, this symptom incidence was	WMV +ve
	SH022	about 1/40, photos Brendon	
		possibly SqMV on squash, leaf margins protruding a	-ve
		little, fruit colour green instead of yellow, incidence of	
	SH023	symptom was 1 in the field	
		paddy melon - combined sample from single plant on	-ve
		field opposite the squash plot and from in between the	
	SH024	old pumpkin/squash plot and the zucchini/squash plots	
	SH025	old squash crop, symptomatic	WMV +ve
	SH026	old squash crop, symptomatic	WMV +ve
	SH027	old pumpkin crop, symptomatic	WMV +ve
5	SM021	sample of typical symptomatic plant	WMV +ve

	SM023	symptomatic paddy melon,	WMV +ve
6	R021-23	possible symptomatic plant	-ve
	R022	possible symptomatic plant	-ve
	R023	possible symptomatic plant	-ve
	R024	possible tospo,	-ve
7	R2021	chlorosis and leaf curling, watermelon	-ve
	R2022	green, leaf curling, vigorous watermelon plant, Denis not impressed	-ve
	R2023	random samples of watermelon, bulks of ten per sample bag	-ve
8	M2021-M2022	yellowing of honeydew plants, possible toxicity or nutritional; inspected on request from owner	-ve
9	M1024	erect and cupped zucchini leaves, photo taken	-ve
	M1025	small stunted zucchini plant, photo taken	-ve
	M1026	mild mosaic on older zucchini leaf, photo taken	-ve
	M1027	random samples of zucchinis, bulked in ten	-ve

a range of symptoms in the crop. Similar results were obtained at property 3 with WMV being detected in all the symptomatic plants tested and this virus was also detected in paddymelon. WMV was detected in zucchini, squash and pumpkin at property 4 and a symptomatic paddymelon tested positive for WMV at property 5. No virus was detected in any of the symptomatic plants sampled on properties 6 – 9 even though the same cropping systems and cultivars of cucurbits were grown on these properties. The most significant difference between Properties 1-5 and properties 6-9 is geographic location. Properties 1-5 were in and around the Swan Hill – Lake Boga irrigation districts, while properties 6 – 9 were located approximately 150 km along the Murray river to the north west near Mildura.

2009 survey – Swan Hill Region (Tuesday- Thursday 10-12/2/09):
In February 2009 five properties growing cucurbits were surveyed in the Swan Hill and Lake Boga district on the basis of the survey results from 2008. The purpose of this survey was twofold:

1. To confirm the trends with respect to virus presence, incidence and disease severity.
2. Conduct more intensive molecular analysis of zucchinis showing a range of symptoms and determine if WMV is the only virus associated with these plants, or if other previously undetected viruses are present.

Table 3 summarises the ELISA results for each property. For a complete set of survey results please refer to appendix 2.

Table 3: Cucurbit crops surveyed, symptoms observed and viruses detected using ELISA in February 2009.

Property	Crop/s	Description	Viruses detected
1	Zucchini (Congo)	high incidence of severe mosaic symptoms	WMV (+ve 20/20) CMV (+ve 4/20)
2	Zucchini (Congo)	Very few and only mild symptoms	WMV (+ve 2/20) CMV (+ve 1/20)
3	Zucchini (Congo)	Mild to moderate mosaic symptoms (5-	WMV

		10% incidence)	(9/20 +ve)
4	Zucchini – Congo (100 leaves) Paydo (100 leaves)	Moderate to severe mosaic symptoms (20%)	WMV (11/20 +ve: 3/10; 8/10)
5	Zucchini (Congo)	Mature crop, high incidence of severe mosaic symptoms	WMV (+ve 20/20)

In general, similar trends for incidence of virus and symptom expression were observed to those obtained in 2008. WMV was the predominant virus detected and there was a correlation between WMV positive ELISA tests and the incidence of disease symptoms in the field. Of interest was the detection of CMV at 2 properties. In each of the 5 CMV positive pooled samples WMV was also detected. There appeared to be no specific symptom associated with the presence of CMV in the zucchini plants. Two paddymelon samples were also tested by ELISA at property 1 and one of these samples tested positive for CMV.
Eight additional zucchini samples were collected from property 1 and each sample was expressing a different symptom type. Table 4 summarises the ELISA and PCR results obtained on each sample.

Table 4: Test results on 8 zucchini and squash samples collected from Property 1 in February 2009

Sample	Symptoms	ELISA Results[1]	PCR results
ZR25	Moderate mosaic, leaf distortion and erect plant form	No virus detected	Potyvirus positive
ZR26	Mild mosaic symptom	No virus detected	Potyvirus positive
ZR27	Severe mosaic and leaf distortion	No virus detected	Potyvirus positive
ZR28	Erect plant form with distinct yellow appearance	No virus detected	Potyvirus positive
ZR29	Aussie Gold symptom on squash fruit	No virus detected	Potyvirus positive
ZR30	Severe mosaic symptoms and slight lumpiness on fruit	No virus detected	Potyvirus positive
ZR31	Severe leaf mosaic and shoestring effect on leaves	No virus detected	Potyvirus positive
ZR32	Leaf mosaic and erect plant form	No virus detected	Potyvirus positive

1. The WMV ELISA was conducted using the "old" WMV antiserum and this test result is therefore unreliable.

A range of virus-specific and group-specific PCR tests were conducted on each of the 8 samples (Table 5). A total of 10 tests were done on the 8 samples. Five of the PCR

Table 5. RT-PCR results from 10 RT-PCR tests done on the 8 Cucurbit samples

		Specific PCRs					Universal PCRs				
							Potyvirus			Carla virus	Tospo virus
Sample ID	Symptoms	CaVY	CeMV	AVY	CMV	HSVd	NIb2F-3R	Poty 1-2	WCIEN		
ZR25	Shoestring	-	-	?	-	-	+	-	+	-	-
ZR26	Severe symptoms	-	-	?	-	-	+	-	+	-	-
ZR27	Erect yellow	-	-	?	-	-	+	-	+	-	-
ZR28	Erect yellow	-	-	?	-	-	+	-	+	-	-
ZR29	Mild	-	-	?	-	-	+	-	+	-	-
ZR30	Aussie Gold squash (mosaic)	-	-	?	-	?	+	-	+	-	-
ZR31	Severe mosaic	-	-	?	-	-	+	-	+	-	-
ZR32	Erect	-	-	?	-	-	+	-	+	-	-

+ Positive
- Negative
? Ambiguous results

tests were genus-specific for the genera *Potyvirus, Carlavirus* and *Tospovirus*. The other 5 tests were species-specific for Carrot virus Y (CaVY), Celery mosaic virus (CeMV), Apium virus Y (AVY), Cucumber mosaic virus (CMV) and Hop stunt viroid (HSVd).

All samples tested positive for potyvirus-specific RT-PCRs using the NIb2F-3R and WCIEN primers. Amplicons from samples ZR27, ZR28 and ZR31 in the NIb2F-3R and WCIEN tests were cloned. Three clones of each successfully cloned fragment were then sequenced in both the forward and reverse direction. The sequences obtained were approximately 350bps and >800bps in length for fragments amplified by the NIb2F-3R and WCIEN primers respectively. BLAST searches were done using the sequences and the results are as below (Table 6). For each cloned amplicon that was sequenced the closest match was with WMV at a sequence homology of 95% or higher.

cDNA fragments approximately 300bp in size were also amplified from all samples tested in the AVY-specific RT-PCR test, although the correct amplicon sizes are expected to be 400bps. The amplicons from samples ZR27, ZR28 and ZR31 were purified and directly sequenced in both the forward and reverse direction. The sequences obtained were approximately 265bps in length and used in BLAST searches against the nucleotide sequence database GenBank (Table 6). The closest match was with WMV with percentage homology of 93% for the ZR27 and ZR28 amplicons and only 84% homology with WMV over 265 bps for the ZR31 amplicon (Table 6).

Table 6. BLAST results of amplicons sequenced from the AVY-specific and potyvirus specific (Nib2F-3R; WCIEN) PCRs on selected cucurbit samples collected from property 1 (February 2009).

Samples	BLAST results		
	AVY	NIb2F-3R	WCIEN
ZR27	230/247 (93%) similar to WMV isolate FMF03-141 (EU660583.1)	322/339 (94%) similar to WMV isolate FMF03-141 (EU660583.1)	690/706 (97%) similar to WMV strain WMV-CHN (DQ399708.1) 679/706 (96%) similar to WMV isolate FMF03-141 (EU660583.1)
ZR28	214/229 (93%) similar to WMV isolate FMF03-141 (EU660583.1)	320/336 (95%) similar to WMV isolate FMF03-141 (EU660583.1)	N/A Host genome amplified
ZR31	153/181 (84%) similar to WMV isolate FMF03-141 (EU660583.1)	N/A	574/589 (97%) similar to WMV strain WMV-CHN (DQ399708.1) 561/589 (95%) similar to WMV isolate FMF03-141 (EU660583.1)

Multiple bands were observed when PCR products from the HSVd-specific RT-PCR test were visualised on the UV-transilluminator after agarose-gel electrophoresis. The positive control yielded a strong single band of 300bp in size. Although bands of the same size were observed from all samples, they were often faint, except the one produced from sample ZR30. However, since multiple size products were amplified from all samples, not like the single cDNA fragment amplified from the positive control, it was concluded that the samples are most likely not infected with HSVd.

cDNA fragments approximately 750bp in size were amplified from all samples except ZR30 in the Tospovirus group-specific RT-PCR test, although the correct amplicon sizes are expected to be 870bps. Sequence results later indicated that the smaller sized bands were due to non-specific binding of the primer to the plant genomic DNA.

All other PCR tests were negative (Table 5).

2010 Survey – Swan Hill Region (16-18/3/2010).

In March 2010 a third and final survey of cucurbit crops from the Swan Hill district were surveyed with the aim of collecting a third year of data on selected properties. Some alternate weed hosts were also targeted for the presence of potyviruses. In addition, talks were also held with cucurbit growers from the Lake Boga and Swan Hill area about management of viruses in cucurbit crops.

Sampling methodology and ELISA testing was the same as in previous years. Where appropriate additional PCR tests were conducted. Table 7 summarises the ELISA results from the 4 properties. For a complete set of survey results please refer to Appendix 3.

Table 7: Cucurbit crops surveyed, symptoms observed and viruses detected using ELISA in March 2010.

Property	Crop/s	Description	Viruses detected
1	Block 1: Zucchini (Congo)	Medium aged crop (just starting to fruit), mild symptoms observed at < 1% incidence	WMV (7/11 +ve)
1	Block 2: Zucchini (Congo and Crowbar)	Congo – 25-30% incidence of moderate symptoms (140 leaves sampled) Crowbar – 0% incidence (100 leaves)	WMV (6/24 +ve)
2	Zucchini - congo	Moderate symptoms observed < 5% incidence	WMV (2/10 +ve)
3	Zucchini - Houdini	No symptoms observed	WMV (1/19 +ve)

A number of additional samples were collected for further analysis using PCR (Table 8). Based on the results from the 2009 survey and the detection of a potyvirus using primers specific to *Apium Virus* Y (ApVY) (for more discussion on this finding see below) samples from weeds in the Apiaceae, particularly fennel (or aniseed weed) were collected. Potyviruses were detected in only 2 of the 11 samples and both these samples were symptomatic Zucchini plants. An additional 3 symptomatic zucchini samples and one symptomatic squash plant tested negative for potyviruses. A total of 5 aniseed weed samples tested negative for both potyviruses and ApVY. These five samples do represent a very small sample of the aniseed weed population and is possible that our RNA extraction procedures are not optimal for extracting RNA from the viscous aniseed sap.

Table 8: Test results on individual samples collected on the March 2010 survey.

Sample	Sample description/Symptoms	Poty PCR	ApVY PCR
1	Prop 1 Old Block Congo Symptoms	**Pos**	Neg
2	Prop 1 Block 1 Zucchini Symptoms	Neg	Neg
3	Prop 1 Block 1 Squash	Neg	Neg
4	Prop 1 Block #2 Aniseed (1)	Neg	Neg
5	Prop 1 Block #2 Aniseed (2)	Neg	Neg
6	Prop 1 Block #2 Aniseed (3)	Neg	Neg
7	Prop 1 Block 2 Aniseed 4	Neg	Neg
8	Prop 2 Zucchini Congo symptoms	Neg	Neg
9	Prop 2 Zucchini Congo symptoms	Neg	Neg
10	Prop 3 Zucchini Congo symptoms	**Pos**	Neg
11	Prop 3 Aniseed plant	Neg	Neg

Conclusions:

WMV was detected at significant levels in Swan Hill but not at Wemen (only 0.5%) or in the Mildura district. The incidence of WMV on some Swan Hill properties was extremely high in both zucchini and squash crops and ranged from <1 % to 80% in some mature crops. SqMV PRSV and ZYMV were not detected in the survey in any of the three surveys conducted. CMV was detected in a total of 4 four samples in the 2009 and 2010 surveys, however there did not appear to be any correlation between the presence of CMV and the symptoms expressed by the zucchini plants.

Paddymelon was found to be infected with WMV on several properties in different years and CMV was detected in paddymelon at property 1. These findings highlight the role this weed can play as an alternate host and a source of inoculum of cucurbit viruses.

In the second year of the surveys there was some confusion generated on the incidence of WMV in field samples due to the source of WMV antiserum used in the ELISA tests. Although frustrating it does reflect potential variation between strains of WMV. Similar experiences with WMV have been reported from the USA Vincelli and Seebold, 2009) who reported the detection of a potyvirus in symptomatic cucurbit crops using the AgDia Inc. Potyvirus group ELISA and which tested negative using the AgDia Inc. CVS test which includes tests for *Cucumber mosaic cucumovirus*, *Impatiens necrotic spot tospovirus*, *Papaya ringspot potyvirus*, *Squash mosaic comovirus*, *Tobacco mosaic tobamovirus*, *Tobacco ringspot nepovirus*, *Tomato mosaic tobamovirus*, *Tomato ringspot nepovirus*, *Tomato spotted wilt tospovirus*, *Watermelon mosaic potyvirus* (WMV) (formerly *Watermelon mosaic virus 2*) and *Zucchini yellow mosaic potyvirus*. RNA was extracted from two symptomatic Potyvirus "positive" pumpkin samples and a potyvirus was amplified using potyvirus-specific primers. Sequence analysis identified a strain of WMV with 95 – 97 % homology with multiple accessions of WMV strains. These results indicate that a strain of WMV is present in Kentucky which does not react to the current AgDia Inc. Tissue samples from the PVG-positive, CVS-negative plants analysed in this study were provided to AgDia Inc., in order to facilitate the development of a WMV-specific ELISA test that will detect this new strain.

There appears to be a poor association of the presence of WMV and symptoms expressed on zucchini plants in the field. The preferred cultivar of Zucchini was Congo and it is possible that this cultivar is more susceptible to WMV than other zucchini cultivars. Symptoms associated in Congo and which only WMV was detected include mild mosaic, mosaic, severe mosaic which included leaf deformation and plant stunting. WMV was also detected in a zucchini plant showing a golden mosaic symptom at Property 1. The zucchini cultivar "Crowbar" generally had a lower incidence of WMV infection (e.g. March 2010 grower 1 Block 2) and this may be an important component of an overall IPM strategy for controlling WMV losses in cucurbits.

WMV was also associated with a range of mosaic symptoms in squash including the fruit symptom referred to as "Aussie Gold". A squash plant that was showing the Aussie Gold symptom on its fruit also tested negative for WMV and none of the remaining viruses was detected in these plants. Symptom variability could partly be due to time of infection, rate of growth etc. However the observed inconsistency may be due to variation within the virus or possibly a second viral pathogen which was not detected with the diagnostic tests used in this study.

Because the correlation between the presence of WMV and symptoms on the zucchini plants was so strong it is difficult to identify additional potyviruses that may be present in the symptomatic zucchini plants as it is likely that the potyvirus specific primers are likely to detect and amplify WMV as confirmed by the sequence data. As an additional screen for other potyviruses we tested the individual samples from Property 1 for the presence of *Apium Virus Y* (ApVY). ApVY is a potyvirus that is thought to be native to Australia and infects native Apiaceae as well as carrot and celery. Interestingly this PCR did generate a band but which was not of the correct size. This amplicons was sequenced and in one isolate yielded only an 84% match with the nearest potyvirus, which happened to be WMV. This result suggests that there is a possibility of a second potyvirus that is infecting the zucchinis and this could explain the range of virus-like symptoms that are observed in the zucchini crops in the Swan Hill area.

Based on these results from the 2009 survey, samples from weeds in the Apiaceae, particularly fennel or aniseed weed were targeted at Property 1 as this property, which is located in the Swan Hill area, consistently had more severe virus symptoms on cucurbits when compared to other zucchini growers in the district. Coincidentally Property 1 uses fennel (or aniseed weed), which is a member of the Apiacaeae as a wind break surrounding his vegetable crops. A small number of fennel samples were collected in the 2010 survey and each sample tested negative for potyviruses using RT-PCR. Despite this result the possibility that these border plants are an alternate host for potyviruses that can infect zucchinis should be examined further. More research is required in this area.

Acknowledgements:
We would like to acknowledge the efforts of Matt and Brian from Swan Hill Chemicals for helping us with grower visits in the Swan Hill district and to Sally-Ann Henderson for her help in the Wemen and Mildura districts. We would also like to thank the growers who welcomed us on to their properties.

References:
Persley, D, Cook, T. and House S. (2010). Diseases of vegetable crops in Australia. CSIRO Publishing. 9780643096387.

Vincelli, P., and Seebold, K. (2009). Report of a Watermelon mosaic potyvirus strain in Kentucky undetected by ELISA. Online. Plant Health Progress doi:10.1094/PHP-2009-0313-01-BR.

Surveys for viruses of Lettuce crops in Victoria (2009 – 2010)

Introduction:
Lettuce is a member of the Asteraceae family and is an annual plant of Mediterranean origin that prefers a relatively cool climate and is grown worldwide as a fresh leafy vegetable. The types of lettuce grown include the traditional types iceberg and Cos, and more recently the Oakleaf, Coral and Monet varieties. Lettuce was Australia's 7th largest vegetable crop in 2007/08, valued at $168.0 million and accounting for 5.0% of total vegetable production by value. Production is concentrated in the Eastern states, particularly Queensland and Victoria, who produce 34% and 32% of the annual crop respectively.

Lettuce are susceptible to a wide range of pests and diseases including bacterial leaf spots, wilt diseases, mildew and *Tomato spotted wilt virus* (TSWV). However a detailed survey of viruses infecting lettuce has not been conducted in Victoria for several years. Here we report the findings of surveys conducted in Werribee, the Mornington Peninsula and East Gippsland in 2009 – 2010.

Methodology:
Survey methods
The method used for the cucurbit survey in Victoria was in line with the sampling and testing range used for similar surveys in Queensland. Accurate data on crop locations, disease incidence and symptoms, cultivars collected and crop age was collected when

ever possible. Symptomatic plants representing the range of symptom types seen were collected as individual additional samples and tested by RT-PCR for *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Lettuce necrotic yellows virus* (LNYV), *Lettuce mosaic virus* (LMV) and *Lettuce big vein virus* (LBVV).

Results and Discussion

East Gippsland Survey (4-5/5/09):
Each farm in the Lindenow vegetable growing district had a similar cropping system. All farms were on the river flats and each property rotated a brassica crop with Lettuce and possibly uses corn as a third crop. Each property grew a wide range of lettuce types including Cos, and salad (Oakleaf and Coral varieties) lettuces as well as endive. Lettuce production in this area is for 12 months of the year and for some of the larger properties there may be enough land to rest the soil between these three crops.

Four properties in the Lindenow Valley (Lindenow/Woodglen area) were surveyed and a total of 16 lettuce samples were collected and screened for a range of viruses using RT-PCR. Table 1 lists the samples collected, symptoms observed and the viruses detected.

LBVV, TSWV and LNYV were detected in the symptomatic lettuce plants collected from the Lindenow vegetable growing district in East Gippsland. LBVV was detected in 6 of the 15 lettuce samples that were showing virus-like symptoms. All six LBVV positives were from Cos lettuce and 5 of the 6 LBVV positive plants were showing symptoms typical of LBVD. Significantly two Cos lettuce samples

Table 1: Lettuce and brassica samples collected from Lindenow (East Gippsland) and Werribee in May 2009 and screened for common viruses using RT-PCR.

Virus	LBVV	CMV	TSWV	LNYV	LMV
Sample name					
1. Property 1:Brassica self-sown in drainage ditch	Tested negative for CaMV and potyviruses using RT-PCR				
2. Property 2: Lettuce (Cos) - ? LBV symptoms	Neg	Neg	POS	Neg	Neg
3. Property 2: (Cos) LBVV symptoms	POS	Neg	POS	?Pos	Neg
4. Property 2 – (Cos) big vein classic symptoms	POS	Neg	POS	POS	Neg
5. Property 2: Lettuce (Oakleaf) - ?TSWV symptoms	Neg	Neg	POS	Neg	Neg
6. Property 2: Lettuce (Coral) - ?TSWV symptoms	Neg	Neg	Neg	Neg	Neg
7. Property 3: Cos Lettuce Classic TSWV symptoms	POS	Neg	POS	Neg	Neg
8. Property 3: Cos lettuce classic big vein symptoms	Neg	Neg	Neg	Neg	Neg
9. Property 3: Cos lettuce, deformed, stunted	?Pos	Neg	Neg	POS	Neg
10. Property 3: (Oakleaf) – classic TSWV symptoms	Neg	Neg	POS	Neg	Neg
11. Property 3: (Coral) - ?TSWV symptoms	Neg	Neg	Neg	Neg	Neg
12. Property 3: Cos lettuce – classic TSWV symptoms	Neg	Neg	Neg	POS	Neg
13. Property 3: Cos lettuce - ? virus	Neg	Neg	POS	Neg	Neg
14. Property 4: Cos lettuce – classic LBVV symptoms, 80% incidence	POS	Neg	POS	Neg	Neg
15. Property 4: Cos lettuce – classic LBVV and TSWV	POS	Neg	Neg	Neg	Neg
16. Property 4: Cos lettuce – LBVV symptoms	POS	Neg	Neg	POS	Neg
17. Prop 3: Cabbage - ?Turnip mosaic virus – do potyvirus PCR	Tested negative for CaMV and potyviruses using RT-PCR				
18. Werribee expo - celery with mosaic symptoms	Neg	POS	POS	Neg[1]	Neg
19. Werribee expo - celery with mosaic symptoms	Neg	Neg	Neg	Neg[1]	Neg
20. Werribee Expo - parsley with mosaic symptoms	Neg	POS	POS	Neg	Neg
Werribee 1.	Pos	Neg	POS	Neg	Neg
2	Pos	POS	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	POS	POS	POS	Neg

[1.] Sample also tested positive for potyvirus – this most likely *Celery mosaic potyvirus*

that were showing LBVD symptoms tested negative for LBVV. This result, and the presence of LBVV in a plant not expressing typical LBVD symptoms (Sample 7) suggests that an additional virus may be associated with LBVD and this requires further investigation. LBVV was not detected in Oakleaf and Coral lettuce even though these plants were grown in an adjacent bay that was expressing LBVD symptoms in Cos lettuce.

TSWV was detected in plants expressing typical TSWV symptoms (necrotic lesions and ringspots on the leaves, general stunting of the infected plant – if infected at a young age) as well as in plants expressing marginal LBVD symptoms (Sample 2). TSWV was detected twice in Oakleaf lettuce cultivars but not in the coral lettuce cultivars. LNYV was detected in several lettuce plants either co-infected with LBVV and/or TSWV or as a single virus infection. LNYV was the only virus detected in Sample 9 which was expressing slightly deformed and stunted appearance. This symptom together with a marginal yellowing of the leaves (particularly on Cos and iceberg lettuce) are the more typical symptoms reported to be associated with this virus.

CMV was not detected in any of the 15 samples collected in this survey. However, in past surveys CMV has been detected in lettuce grown in East Gippsland (data not shown).

Four lettuce samples were collected from the Werribee "Expo" Field Day experimental plots (May 2009) and all four viruses were detected either as single virus or mixed infections of LBVV, TSWV, LNYV and CMV (Table 1, Werribee 1 – Werribee 4). The field plots were planted by a range of commercial companies as demonstration plots and clearly indicated that an inoculum source for each of these viruses is readily available in the Werribee vegetable growing district. A more detailed survey of Vegetable farms in this area was conducted in December 2009.

Two brassica samples (Sample 1, 17) were collected from the Lindenow district and screened for potyviruses and *Cauliflower mosaic virus* and both samples were negative. This is not a clear representation of the incidence of viruses in brassicas and more extensive surveys of brassica crops in Victoria is required to determine the incidence of viruses in brassica crops.

Survey of a Mornington Peninsula lettuce farm (10/11/09)

On 10/11/09 the research team (Brendan Rodoni and Liz Minchington) visited a lettuce grower on the Mornington Peninsula who was experiencing significant lettuce big vein disease (LBVD) problems. In some crops classic symptoms of LBVD were present on greater than 80% of the plantings, particularly in the older crops (planted late September).

There were two varieties of lettuce and variation in susceptibility to LBVD was observed. "Catalin" was the most common cultivar and was more tolerant to virus infection and "Silverado" which was a trial cultivar and found to be very susceptible to disease symptoms. Table 2 lists the samples collected and the RT-PCR results for five viruses known to infect lettuce in Victoria. Each sample was tested for *Lettuce big vein* virus (LBVV) that is associated with lettuce big vein disease (LBVD), *lettuce necrotic*

yellows virus (LNYV), *Cucumber mosaic virus* (CMV), *Lettuce mosaic virus* (LMV) and *Tomato spotted wilt virus* (TSWV).

Table 2: Lettuce samples collected from a Mornington Peninsula lettuce farm in November 2009 and screened for five viruses using RT-PCR.

Sample:	**LBVV**	**LNYV**	**CMV**	**LMV**	**TSWV**
1. Old plants - LBVD symptoms	**Pos**	Neg	**Pos**	Neg	Neg
2. Old plants - LBVD and yellow leaves	**Pos**	**Pos**	**Pos**	Neg	Neg
3. LBVD, yellowing and possible TSWV	**Pos**	Neg	**Pos**	Neg	Neg
4. Old plant - "washed" yellow leaves and maybe LBDD	**Pos**	**Pos**	**Pos**	Neg	Neg
5. Old plant - LBDD and chlorosis on marginal leaves	**Pos**	**Pos**	**Pos**	Neg	Neg
6. 2nd planting (Catalin) classic LBDD	**Pos**	**Pos**	**Pos**	Neg	Neg
7. 2nd planting (Catalin) stunted and yellow plant	**Pos**	**Pos**	**Pos**	Neg	Neg
8. 2nd planting (Catalin), plant – yellowing symptom	**Pos**	**Pos**	**Pos**	Neg	Neg
9. 3 week old seedling with ?LBDD	Neg	Neg	Neg	Neg	Neg
10. Cos Lettuce with LBDD and mosaic	**Pos**	**Pos**	**Pos**	Neg	Neg

The symptoms associated with the range of virus infections are presented in the figures 1 and 2.

The majority of the symptomatic lettuce plants at this property were infected with LBVV, LNYV and CMV. Each virus is a significant virus in their own right and can cause symptoms as single infections. The presence of LNYV was a concern at this property as the negative impacts of this virus can easily be managed by controlling sow thistle. This weed is an important alternate host of both the virus and the aphid vector and was present at a high incidence on this farm. CMV is a widely distributed virus in south east Australia and has a very wide host range and is transmitted by a number of aphid species. Aphids migrating on to the lettuce crop are likely to be the source of inoculum.

LBVV is transmitted by a soil-borne fungus/weak pathogen, *Olpidium spp.* and is one of several viruses associated with lettuce big vein disease (LBVD). Further work is required to identify other viruses that have been reported to be associated with this disease. A likely control strategy is to increase the rotation between lettuce crops as this will reduce the inoculum of virus carrying *Olpidium* spores in the soil. Any plant infected with 3 different viruses is severely compromised and it is likely that these lettuce plants performed poorly, succumbed to other diseases or abiotic stresses.

The lettuce grower at this property did discuss how the shape and performance of the lettuces was affecting the marketability of the lettuce heads. It is difficult to pinpoint virus the cause of these observations but it is feasible that the virus infection was related to the "collapse" or "opening out" of the lower leaves which tend to fall away from the heart and lay on the ground. This causes some problems with marketing as dust/mud gets into the leaves. The virus-infected plants are also more brittle and the hearts do not fill out.

Sample 9 which was a 3 week old lettuce seedling that tested negative for all five viruses. This indicates that the source of inoculum for each virus, including LBVV, is from the farm environment; *Olpidium spp.* infested soils for LBVV and alternate hosts for LNYV, CMV and TSWV.

A further two lettuce samples were submitted from the same property in January 2010 and were expressing symptoms typical of LBVV and a marginal yellowing of the older leaves. Both samples tested positive for LNYV and LBVV and negative for TSWV, CMV and LMV.

Survey of Werribee south Lettuce Farms (15/12/2009)

Two farms at Werribee South were surveyed for lettuce viruses in December 2009. Gatlan was the main cultivar grown on both farms. The grower at property 1 had reported leaving 50% of his previous lettuce crop (Sample 5) in the ground due to chronic LBVD symptoms.

A total of 7 samples were collected from the two properties and tested for TSWV, LNYV, CMV, LBVV and LMV using RT-PCR. The symptoms observed and RT-PCR results are presented in Table 1.

Table 1: Lettuce samples collected from Werribee South in December 2009 and screened for five viruses using RT-PCR.

Virus	TSWV	LNYV	LBVV	CMV	LMV	
Sample name						
1 – Prop 1: Gatlan sample showing classic LBVD	Neg	Neg	**POS**	Neg	Neg	
2 – Prop 1: trial cvr showing LBVD symptoms	Neg	Neg	**POS**	Neg	Neg	
3 – Prop 1: Trial cvr "26" showing severe LBVD symptoms	Neg	Neg	**POS**	Neg	Neg	
4 – Prop 1: Yellows type symptom	Neg	**POS**	**POS**	Neg	Neg	
5 – Prop 1: chronic LBVD symptoms including poor forming hearts	Neg	Neg	**POS**	Neg	Neg	
6 – Prop 2: Trial cvr 27 showing LBVD symptoms	Neg	Neg	**POS**	Neg	Neg	
7 – Prop 2: Severe LBVD symptoms	Neg	Neg	**POS**	Neg	Neg	

LBVD is the major virus disease of concern in Werribee South. Although this was only a small survey the results indicate that the big vein symptom does have an association with LBVV. Comments from both the growers and the Industry development Officer was that the incidence and severity of LBVD was patchy and varied between farms and between years.

Conclusions:

LBVD is the major virus disease of lettuces in Victoria and the economic impact warrants further investigation as does the etiology of the disease. In the surveys conducted in this study LBVV detection was used as indicator for the presence of this disease. It is recommended that a more thorough investigation of all the viruses that may be associated with this disease in Victoria be conducted to determine the true etiology of this syndrome. A taxonomic analysis of the *Olpidium spp.* present in each district/farm would also be worthwhile. It may be that some of the variation in symptom severity both between and within districts is due to presence/absence of one or more viruses and the efficiency of the fungal vector.

Some additional issues on LBVD from a Victorian perspective include:

- Cultivar susceptibility to the disease. This is the main control strategy employed by growers and it is poorly understood.
- The influence on season/climate on the expression of symptoms/disease
- Post harvest "fitness" of LBVD infected plants/leaves, particularly with respect to packaging of "Baby Cos" plants.

A ring necrosis disease associated with viruses transmitted by the fungus *Olpidium* also caused concern in several hydroponic production sites in NSW and Victoria.

In most instances it was difficult to identify either LNYV or CMV in the lettuce plants based on symptoms alone and our estimates of the incidence of these pathogens would have been much lower if we did not test for their presence using RT-PCR. There appears to be variation in symptoms between cultivars, but regardless of cultivar all plants show a range of symptoms within a planting. It is extremely difficult to estimate the economic impact of the lettuce viruses detected in this survey at this point. It is likely to be significant.

LMV was not detected in any of the samples tested and is a clear indication that the growers/nurseries are sowing certified seed as the usual source of inoculum of this virus is from seed.

A lack of thrips activity this spring is the most likely reason for not seeing or detecting TSWV.

Ute guides for "Lettuce Best Practice – Integrated Pest Management" and "Brassica Best Practice – Integrated Pest Management"

Brendan Rodoni provided science input (Virology) into the production of the "Lettuce Best Practice – Integrated Pest Management" and "Brassica Best Practice – Integrated Pest Management" Ute Guides produced by Vic DPI Farm Services Victoria and Queensland DEEDI (editors Rob Dimsey - Vic DPI, David Carey – Qld DEEDI; Sally-Ann Henderson – Vic DPI). The purpose of the guides is to outline the key issues that should be considered in relation to the implementation of Integrated Pest Management (IPM) for lettuce and brassica crops. The guide addresses the key control methods to use within an IPM strategy for the major insect pests, pathogens (including viruses), nematode and weed control. Advice on epidemiology and control for *Lettuce necrotic yellows virus*, *Tomato spotted wilt virus*, *cucumber mosaic virus*, *Lettuce mosaic virus*, *Turnip mosaic virus* and Lettuce big vein disease for Lettuce and for brassicas, *Cauliflower mosaic virus*, *Turnip mosaic virus* and *Beet western yellows virus* was provided.

The effects of climate change on plant virus epidemics of vegetables in Australia

Kyla Finlay, Jo Luck, Denis Persley and Brendan Rodoni.

A draft manuscript has been prepared on the effects of climate change on plant virus epidemics of vegetables in Australia (Appendix 3).

The potential impacts of climate change on plant virus epidemics in food crops has been poorly studied. Given that the Australian vegetable industry was estimated to be worth approximately $3.1 billion dollars in 2006/07 (ABS 2008) this is a serious knowledge gap. The aim of this paper is to review potential impacts of climate change on vegetable viruses in Australia with a view to enhancing the capacity of the vegetable industry to

implement integrated virus disease management programs and ultimately reduce the potential economic impact of climate change.

For Australia the best estimate for annual average temperature increases by 2030 are 1°C with slightly less (0.7° to 0.9°C) in the coastal south and north east and slightly more (1°C to 1.2°C) inland. (CSIRO & BoM 2007). It is predicted that these changes will result in an increase in daily temperature extremes, more extreme weather events and a reduction in rainfall in southern and eastern Australia. It is difficult to predict how these changes will affect plant virus epidemics in vegetable crops. Plant virus epidemics are more likely when plants are exposed to environments where the viruses have not co-evolved with the wild ancestors of those plants (Jones 2009) and climate change is likely to promote this phenomenon as changes in temperature, rainfall and frost frequency will alter the current geographic range of crops, weeds and insect vectors.
The three host/virus/vector scenarios discussed in this paper highlight how difficult it will be to predict the impact of a changing environment on plant virus disease epidemics in vegetables. It is possible for example that existing resistant cultivars could be affected as some R genes fail or are less effective under high temperatures (e.g. above mid 30s). Another possibility is that crops, particularly irrigated vegetable crops, could be more vulnerable to virus infection as they are the green islands in a dry landscape attracting migrating insect populations. The extended production cycles of many vegetable crops that will result in earlier plantings because it is warmer and there are less frosts, will reduce the length of production breaks and therefore enhance the green bridge for viruses and their insect vectors.

It is clear that cropping systems, insect vector incidence and the abundance of alternate hosts for viruses and insect vectors will change with climate change. To counteract increased losses in vegetable production it is imperative that each cropping system be carefully monitored for the emergence of new plant disease epidemics.

Miscellaneous activities:
Sydney Basin Survey (20-22/4/09)
Brendan Rodoni participated in a survey of vegetable crops in the Sydney Basin in April 2009. During this 3 day field trip a range of TSWV symptoms were observed on tomatoes and lettuces and suggested that different TSWV strains may be responsible for the range of symptoms observed. There is a lack of full length sequence data of TSWV isolates in Australia and this may be a worthwhile research activity in the near future.

A yellows symptom on glasshouse grown cucumbers was sampled for further testing. The initial symptom is a chlorotic spot on the leaf, which progresses to a distinct mosaic and finally in older leaves it causes inter-veinal chlorosis, which looks like netting. It was suggested that the spread of this disease is linked to whiteflies and it is not uncommon to have 100% incidence of these symptoms in the glasshouse. This is understandable given that each polyhouse grows the cucumbers under a continuous cropping system, with the seedlings of the next cucumber crop planted in the coconut peat bags before the previous crop is finished bearing fruit. The symptoms did look viral related and the association with whiteflies as the vector suggested that a begomovirus or crinivirus may be associated with these plants. RT-PCR tests were

conducted in the Queensland DEEDI labs and tested positive for *Beet pseudoyellows virus*.

Canarvon Field Trip (September 2010).
Brendan Rodoni participated in a field trip of the Canarvon region (Western Australia) looking at cucurbit viruses with Brenda Coutts

Appendix 1. Property and sample description and ELISA results for cucurbit samples collacted in the Swan Hill Mildura region in March 2008.

Property 1: (Speewa Punt Rd; Lat 35 25205, Long 143 52177)

Crops

Crop	**Plant Age**	**Symptoms**	**Incidence**
Squash Sunburst	Fruiting (2 bays, 8 rows per bay, 200 plants per row)	severe, leaf & fruit	<1%
Zucchini Congo	Fruiting (6 bays, 8 rows/bay, 200 plants/row)	severe	<1%

Weeds

Weeds	**Plant age**	**Location**	**Prevalence**
Caltrope	Fruiting	Field edge only	rare

Samples	**Description**	**Virus Detected**
TF001-TF020	random samples of zucchini, bulks of ten per sample bag	4/20 WMV +ve
TF021	Aussie Gold squash, symptomatic, fruit in same bag but from different plant same leaf symptoms;	-ve
TF022	single zucchini sample	-ve
TF023	Aussie gold squash	WMV +ve
TF024	PRSV-like symptoms in zucchini	WMV +ve
TF025	erect, yellow symptom in zucchini	-ve
TF026	odd, possible nutritional	-ve
TF027	individual plant sample of squash;	-ve

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
TF001 1	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	**POS**
6	Neg	Neg	Neg	Neg	**POS**
7	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg

20	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg
22	Neg	Neg	Neg	Neg	Neg
23	Neg	Neg	Neg	Neg	**POS**
24	Neg	Neg	Neg	Neg	**POS**
25	Neg	Neg	Neg	Neg	Neg
26	Neg	Neg	Neg	Neg	Neg
27	Neg	Neg	Neg	Neg	Neg

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 4/20 of the pooled samples of zucchini and equates to an estimate of 2.15% levels of WMV in the Zucchini crop.

Of the individual plants that were sampled TF021-TF027) there were some interesting results. WMV was detected in a Zucchini displaying the "Aussie gold" symptom (TF023) while no virus was detected in another sample (TF021) showing the same symptom. WMV was detected in a second zucchini plant showing severe leaf distortion, a symptom more typical of those associated with PRSV (D. Persley, pers comm.).

Property 2: (Woomien Rd; Lat 35.25307, Long 143.49863).

Crop	Plant age	Symptoms	incidence
Zucchini Congo	senescing (8 bays, 12 rows/bay, 250 plants/row)	severe-very severe	>95%

Planting - 2 crops per year, this crop was planted in October, next one is just emerging, there is a break of cropping over winter

Weeds	Plant age	Location	Prevalence
night shade	flowering	within and around crop	many
paddy melon		within and around crop	few
others?			

Samples	**Description**	**Virus Detected**
MB001-020	random samples, bulks of ten per sample bag	20/20 +ve WMV
MB021	paddy melon possible symptoms	WMV +ve
MB022	mosaic,	WMV +ve
MB023	golden mosaic symptom,	WMV +ve

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
MB001 1	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	**POS**
3	Neg	Neg	Neg	Neg	**POS**
4	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	Neg	**POS**
6	Neg	Neg	Neg	Neg	**POS**
7	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	**POS**
9	Neg	Neg	Neg	Neg	**POS**
10	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	Neg	**POS**
14	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	**POS**
MB001 18	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	**POS**
21	Neg	Neg	Neg	Neg	**POS**
22	Neg	Neg	Neg	Neg	**POS**
23	Neg	Neg	Neg	Neg	**POS**

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 20/20 of the pooled zucchini samples and this equates to an estimate of 31.75% levels of WMV incidence in the crop. It is likely that the true incidence is higher.

A paddymelon was found to be infected with WMV (MB021) and this highlights the role this weed can play as an alternate host of WMV and a source of inoculum.

Only WMV was detected , even though there were a range of symptoms in the crop. It is possible that a second virus is associated with some of these symptoms. More research is required.

Property 3: Lake Rd, Lake Boga; Lat 35.47261, Long 143.6004

Crop	Plant age	Symptoms	incidence
Zucchini Congo	fruiting	moderate	11/200
	(132 rows at 350 plants per row)		
Zucchini Congo	vegetative	healthy	0/300
	(34 rows at 350 plants per row)		

Weeds	Plant age	Location	Prevalence	Symptoms
paddy melon	Fruiting symptoms on some only	within and around crop	abundant	mild
Caltrope	Fruiting	field edge only	many	healthy
helitrope	flowering	field edge only	rare	healthy

Samples	Description	Virus Detected
SH2001-SH2020	random samples, bulks of ten per bag; SH2001 & SH2002 of the younger zucchini crop	7/20 WMV +ve
SH2021	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2022	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2023	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2024	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2025	paddy melon random sample of bulk ten plants collected from younger zucchinis	WMV +ve
SH2026	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2027	symptomatic plant from the oldest zucchini bays	WMV +ve
SH2028	paddy melon random sample of plants collected from headland adjacent to oldest zucchini planting	WMV +ve

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
SH1001 001	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	**POS**
4	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	**POS**

20	Neg	Neg	Neg	Neg	**POS**
21	Neg	Neg	Neg	Neg	**POS**
22	Neg	Neg	Neg	Neg	**POS**
23	Neg	Neg	Neg	Neg	**POS**
24	Neg	Neg	Neg	Neg	**POS**
25	Neg	Neg	Neg	Neg	**POS**
26	Neg	Neg	Neg	Neg	**POS**
27	Neg	Neg	Neg	Neg	**POS**
28	Neg	Neg	Neg	Neg	**POS**

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 7/20 of the pooled samples and equates to an estimated level of 4.00% WMV in the Zucchini crop.

All symptomatic plants tested positive for WMV.

WMV was detected in paddymelon which highlights the importance of this weed as an alternate host for this virus.

Property 4: Cumnock Rd, Tresco, Swan Hill; Lat 35.49879, Long 143.64055

Crop	Plant age	Symptoms	incidence
Zucchini Congo	fruiting 36 rows at 180 plants per row; 8-10 weeks old	moderate-severe	>95%
pumpkin/squash	senescing (pumpkins 90 days old, plot was located between zucchini's and squash,)		
Squash sunburst	fruiting (two symptom types, 8 rows with 40 plants per row, all inspected, 4-5 weeks old; second squash plot present but not inspected)	mild	low, 1/40 & 1/300

Weeds	Plant age	Location	Prevalence
paddy melon		field edge only	rare
caltrope	Fruiting	within and around field	many
helitrope	flowering	within and around field	many
portulaca	flowering	within and around field	rare

aphids observed on one plant by Matt but presence not actively surveyed for

Samples	Description	Virus Detected
SH001=SH020	random samples collected from zucchinis, bulked ten plants per sample	20/20 WMV +ve
SH021	PRSV-like symptom, severe and typical for the crop, light-dark green mosaic, h/r no shoe-string symptom	WMV +ve

SH022	mosaic symptom on squash, this symptom incidence was about 1/40, photos Brendon possibly SqMV on squash, leaf margins protruding a little, fruit colour green instead of yellow, incidence of symptom was 1 in the field	WMV +ve
SH023	paddy melon - combined sample from single plant on field opposite the squash plot and from in between the old pumpkin/squash plot and the zucchini and squash plots	
SH024	old squash crop, symptomatic	WMV +ve
SH025	old squash crop, symptomatic	WMV +ve
SH026	old pumpkin crop, symptomatic	WMV +ve
SH027		

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
SH001 1	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	**POS**
3	Neg	Neg	Neg	Neg	**POS**
4	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	Neg	**POS**
6	Neg	Neg	Neg	Neg	**POS**
7	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	**POS**
9	Neg	Neg	Neg	Neg	**POS**
10	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	**POS**
SH001 13	Neg	Neg	Neg	Neg	**POS**
14	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	**POS**
18	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	**POS**
21	Neg	Neg	Neg	Neg	**POS**
22	Neg	Neg	Neg	Neg	**POS**
23	Neg	Neg	Neg	Neg	Neg
24	Neg	Neg	Neg	Neg	Neg
25	Neg	Neg	Neg	Neg	**POS**
26	Neg	Neg	Neg	Neg	**POS**
27	Neg	Neg	Neg	Neg	**POS**

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 20/20 of the pooled samples and equates to an estimate of 31.75% level of WMV infection in the crop. It is likely that the true levels of infection are higher.

WMV was detected in Zucchini, squash and pumpkin at this property.

All "old plants" tested were positive.

Property 5: Opposite Mallee Fresh, Swan Hill; Lat 35.27918, Long 143.45567

Crop	Plant age	Symptoms	Incidence
Zucchini – Congo	Fruiting (4 bays, 10 rows/bay, 280 plants/bay)	moderate	4/50 eastern edge, 21/50 middle of block

Weeds	plant age	location	prevalence	symptoms
paddy melon	vegetative age	field edge	many	mild
caltrope	flowering	field edge	many	
bathurst burrs	fruiting	within and around field	rare	

Samples	Description	Virus Detected
SM001-SM020	random samples of watermelon, bulks of ten per sample bag	20/20 WMV +ve
SM021	sample of typical symptomatic plant	WMV +ve
SM023	symptomatic paddy melon,	WMV +ve

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
SM001	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	**POS**
3	Neg	Neg	Neg	Neg	**POS**
4	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	Neg	**POS**
6	Neg	Neg	Neg	Neg	**POS**
SM001 7	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	**POS**
9	Neg	Neg	Neg	Neg	**POS**
10	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	Neg	**POS**
14	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	**POS**

18	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	**POS**
21	Neg	Neg	Neg	Neg	**POS**
Missing					
23	Neg	Neg	Neg	Neg	**POS**

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 20/20 of the pooled samples and represents 31.75% levels of WMV in the Zucchini crop. It is likely that the levels of WMV are higher in this crop.

A symptomatic paddymelon tested positive for WMV.

Property 6: C152 Highway, Wemen; Lat 34.77237 Long 142.6535

Crop	Plant age	Symptoms	incidence
Zucchini Congo	Fruiting (8.5 bays, 8 rows/bay, 300 plants/row)	mild	<1%
Weeds			

No paddy melon, very clean crop, field photos taken on Denis's camera

Samples	**Description**	**Virus Detected**
R001-R020	random samples of zucchini, bulks of ten per sample bag	1/20 +ve WMV
R021-23	possible symptomatic plant	
R022	possible symptomatic plant	
R023	possible symptomatic plant	
R024	possible tospo,	

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
R001 1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg

12	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg
22	Neg	Neg	Neg	Neg	Neg
23	Neg	Neg	Neg	Neg	Neg
24	Neg	Neg	Neg	Neg	Neg

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
CMV, SqMV, PRSV and ZYMV were not detected in any samples tested.

WMV was detected in 1/20 of the pooled samples and equates to 0.5% infection levels of WMV in the crop.

No virus was detected in any of the symptomatic plants sampled.

Property 7: Ryan Road section, Wemen; Lat 34.79051, Long 142.62776

Crop	Plant age	Symptoms	incidence
Watermelon	senescing huge block, >1000 plants inspected	healthy	-
rockmelon	Fruiting huge block, didn't sample, no virus-like symptoms observed	healthy	-

Weeds
Paddy melons dug out but were in and around the watermelon crop, rarely found in general very low weed population, clean crop

Samples	**Description**	**Virus Detected**
R2001-R2020	random samples of watermelon, bulks of ten per sample bag	
R2021	chlorosis and leaf curling, watermelon	
R2022	green, leaf curling, vigorous watermelon plant, Denis not impressed	
R2023	random samples of watermelon, bulks of ten per sample bag	

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
R2001 1	Neg	Neg	Neg	Neg	Neg

R2001 2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg
22	Neg	Neg	Neg	Neg	Neg
23	Neg	Neg	Neg	Neg	Neg

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
No virus was detected on this property.

Property 8: Johns Way, Kardock, Vic; Lat 34.35783, Long 142.34148

Crop	Plant age	Symptoms	incidence
honeydew/rockmelons	Fruiting	healthy	-

6 bays, 5 rows/bay, ~200 plants/row; this was a sub-section. we sampled of a block about 300 acres in size; total production area was 850 acres

honeydew was honey babe aka sweet success and rockmelon was dubloon & plabo

Weeds
very few weeds, very clean property
property very isolated from other cropping areas

Samples	Description	Virus detected
M2001 – M2020	random samples of melons, bulks of ten per sample bag	
M2021-M2022	yellowing of honeydew plants, possible toxicity or nutrional; inspected on request from owner	

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
M2001 1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg
22	Neg	Neg	Neg	Neg	Neg

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
No Virus detected on the property.

Property 9: Trentham, NSW; Lat 34.21402 Long 142.25487

Crop	Plant age	Symptoms	incidence
capsicum - plato	Fruiting sampled, variety 156 also grown but not inspected, also resistant	mild	<1%
sweet chilli - hombre	Fruiting sampled, photos on Denis's camera; adjacent to capsicum, susceptible	severe	10-20%
eggplant	Fruiting not inspected closely but grown close to capsicums and chillies	healthy	-
zucchini - caledia	Fruiting resistant variety, 54 rows, 385 plants/row	healthy	-

Weeds

not many weeds, occasional paddy melon, very clean property; a few helitrope plants in and around field

Samples	Description	Virus Detected
M1001-M1020	random samples of zucchinis, bulked in ten	
M1024	erect and cupped zucchini leaves, photo taken	
M1025	small stunted zucchini plant, photo taken	
M1026	mild mosaic on older zucchini leaf, photo taken	
M1027	random samples of zucchinis, bulked in ten	
M1021	individual sample of symptomatic chilli plant	TSWV +ve
M1022	individual sample of symptomatic capsicum plant	Alfalfa mosaic virus
M1023	individual sample of symptomatic chilli plant	TSWV +ve

Virus	CMV	SqMV	PRSV	ZYMV	WMV
Sample name					
M1001 1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg
Missing	Neg	Neg	Neg	Neg	Neg
Missing	Neg	Neg	Neg	Neg	Neg
Missing	Neg	Neg	Neg	Neg	Neg
24	Neg	Neg	Neg	Neg	Neg
25	Neg	Neg	Neg	Neg	Neg
26	Neg	Neg	Neg	Neg	Neg
27	Neg	Neg	Neg	Neg	Neg

CMV = cucumber mosaic virus; SqMV = squash mosaic virus;
PRSV = papaya ringspot virus; ZYMV = zucchini yellow mosaic virus;
WMV = watermelon mosaic virus

Summary of test results:
No virus detected in the zucchinis surveyed.

Denis Persley detected TSWV in the susceptible chilli plants. TSWV was not detected in the resistant capsicum lines, but Denis did detect alfalfa mosaic virus in the capsicum plants. Denis suggested that next season you do not grow your TSWV susceptible chillies next to your resistant capsicums as this does increase the "virus pressure" on your resistant capsicums.

Appendix 2: Property and sample description and ELISA results for cucurbit samples collacted in the Swan Hill Mildura region in March 2009.

Property 1: (Speewa Punt Rd; Lat 35 25205, Long 143 52177)
(tested2008 – property 1)
Inspected one patch of zucchinis – the cultivar was Congo. The symptoms were more severe on this property and more varied in Range. 200 leaf sample and individual leaf samples collected.

ELISA Results						
Virus	PRSV	ZYMV	WMV	CMV	SqMV	WMV new
Fazz 1	Neg	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	Neg	Neg	**POS**
3	Neg	Neg	Neg	**POS***	Neg	**POS**
4	Neg	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	**POS***	Neg	**POS**
6	Neg	Neg	Neg	Neg	Neg	**POS**
7	Neg	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	Neg	**POS**
9	Neg	Neg	Neg	**POS***	Neg	**POS**
10	Neg	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	**POS***	Neg	**POS**
14	Neg	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	Neg	**POS**
18	Neg	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	Neg	**POS**
ZR 25	Neg	Neg	Neg	Neg	Neg	NT
26	Neg	Neg	Neg	Neg	Neg	NT
27	Neg	Neg	Neg	Neg	Neg	NT

28	Neg	Neg	Neg	Neg	Neg	NT
29	Neg	Neg	Neg	Neg	Neg	NT
30	Neg	Neg	Neg	Neg	Neg	NT
32	Neg	Neg	Neg	Neg	Neg	NT
31	Neg	Neg	Neg	Neg	Neg	NT
32	Neg	Neg	Neg	Neg	Neg	NT
Paddy melon 1	Neg	Neg	Neg	**POS***	Neg	NT
2	Neg	Neg	Neg	Neg	Neg	NT

Property 2: (Woomien Rd; Lat 35.25307, Long 143.49863).
(Tested 2008 – was property 2)

Very few symptoms. Congo was the cultivar. 200 leaf sample and individual leaf samples collected

ELISA Results						
Virus	PRSV	ZYMV	WMV	CMV	SqMV	WMV new
M1	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	**POS***	Neg	**POS**
8	Neg	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg	Neg

Property 3: Lake Rd, Lake Boga; Lat 35.47261, Long 143.6004
(Property tested in 2008 = Property 3)

Similar symptoms to last year, maybe slightly higher incidence. Congo, main cultivar. 200 leaf sample and individual leaf samples collected.

ELISA Results						
Virus	PRSV	ZYMV	WMV	CMV	SqMV	WMV new
TH 1	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg	**POS**
10	Neg	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	Neg	**POS**
18	Neg	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	Neg	Neg

Property 4: Lake Boga WP053 S35.51368 E143.68335 (not tested in 2008)

Higher incidence of symptoms and more severe than at Property 1 (2009). 200 leaf sample and individual leaf samples collected. 100 leaves were from congo, 100 leaves were from Pay-do (Is meant to be resistant)

ELISA Results						
Virus	PRSV	ZYMV	WMV	CMV	SqMV	WMV new
GC 1	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	**POS**
7	Neg	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	**POS***	Neg	**POS**
9	Neg	Neg	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	**POS***	Neg	Neg
12	Neg	Neg	Neg	**POS***	Neg	**POS**
13	Neg	Neg	Neg	**POS***	Neg	**POS**

14	Neg	Neg	Neg	**POS***	Neg	**POS**
15	Neg	Neg	Neg	**POS***	Neg	**POS**
16	Neg	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	Neg	**POS**
18	Neg	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	**POS***	Neg	**POS**

Property 5: Opposite Mallee Fresh, Swan Hill; Lat 35.27918, Long 143.45567 (Tested2008 - property 5)

Old patch of congo and high incidence of symptoms, some severe. 200 leaf sample and individual leaf samples collected. Denis also collected 2 bags of paddymelon. Would be nice to sequence WMV isolate from this tissue.

ELISA Results						
Virus	PRSV	ZYMV	WMV	CMV	SqMV	WMV new
Sam M 1	Neg	Neg	Neg	Neg	Neg	**POS**
2	Neg	Neg	Neg	**POS***	Neg	**POS**
3	Neg	Neg	Neg	Neg	Neg	**POS**
4	Neg	Neg	Neg	Neg	Neg	**POS**
5	Neg	Neg	Neg	Neg	Neg	**POS**
6	Neg	Neg	Neg	**POS***	Neg	**POS**
7	Neg	Neg	Neg	Neg	Neg	**POS**
8	Neg	Neg	Neg	Neg	Neg	**POS**
9	Neg	Neg	Neg	Neg	Neg	**POS**
10	Neg	Neg	Neg	Neg	Neg	**POS**
11	Neg	Neg	Neg	Neg	Neg	**POS**
12	Neg	Neg	Neg	Neg	Neg	**POS**
13	Neg	Neg	Neg	Neg	Neg	**POS**
14	Neg	Neg	Neg	Neg	Neg	**POS**
15	Neg	Neg	Neg	Neg	Neg	**POS**
16	Neg	Neg	Neg	Neg	Neg	**POS**
17	Neg	Neg	Neg	Neg	Neg	**POS**
18	Neg	Neg	Neg	Neg	Neg	**POS**
19	Neg	Neg	Neg	Neg	Neg	**POS**
20	Neg	Neg	Neg	Neg	Neg	**POS**

Appendix 3: **Property and sample description and ELISA results for cucurbit samples collected in the Swan Hill Mildura region in March 2010.**

Property 1: S35.25687 E143.51825 (Property 1 – 2008, 2009)
Block #1: Zucchini – Congo 5-6 weeks old fruiting, mild symptoms, <1% incidence

ELISA Results							
Virus	WMV2	CMV	PRSV	ZYMV	SqMV	WMV new	
Tony Old block 1	Neg	Neg	Neg	Neg	Neg	**POS**	
2	Neg	Neg	Neg	Neg	Neg	**POS**	
3	Neg	Neg	Neg	Neg	Neg	**POS**	
4	Neg	Neg	Neg	Neg	Neg	**POS**	
5	Neg	Neg	Neg	Neg	Neg	**POS**	
6	Neg	Neg	Neg	Neg	Neg	**POS**	
7	Neg	Neg	Neg	Neg	Neg	**POS**	
8	Neg	Neg	Neg	Neg	Neg	Neg	
9	Neg	Neg	Neg	Neg	Neg	Neg	
10	Neg	Neg	Neg	Neg	Neg	Neg	
11	Neg	Neg	Neg	Neg	Neg	Neg	

Block #2:
Congo Zucchini, - 25-30% incidence (140 leaves collected)
Crowbar Zucchini - were located adjacent to Congo, 0% incidence of virus symptoms (100 leaves collected0

ELISA Results							
Virus	WMV2	CMV	PRSV	ZYMV	SqMV	WMV new	
Tony block 1	Neg	Neg	Neg	Neg	Neg	Neg	
2	Neg	Neg	Neg	Neg	Neg	Neg	
3	Neg	Neg	Neg	Neg	Neg	Neg	
4	Neg	Neg	Neg	Neg	Neg	Neg	
5	Neg	Neg	Neg	Neg	Neg	Neg	
6	Neg	Neg	Neg	Neg	Neg	Neg	
7	Neg	Neg	Neg	Neg	Neg	Neg	
8	Neg	Neg	Neg	Neg	Neg	Neg	
9	Neg	Neg	Neg	Neg	Neg	Neg	
10	Neg	Neg	Neg	Neg	Neg	Neg	
11	Neg	Neg	Neg	Neg	Neg	Neg	
12	Neg	Neg	Neg	Neg	Neg	Neg	
13	Neg	Neg	Neg	Neg	Neg	Neg	
14	Neg	Neg	Neg	Neg	Neg	Neg	
15	Neg	Neg	Neg	Neg	Neg	Neg	
16	Neg	Neg	Neg	Neg	Neg	Neg	
17	Neg	Neg	Neg	Neg	Neg	**POS**	
18	Neg	Neg	Neg	Neg	Neg	**POS**	
19	Neg	Neg	Neg	Neg	Neg	**POS**	
20	Neg	Neg	Neg	Neg	Neg	**POS**	

21	Neg	Neg	Neg	Neg	Neg	**POS**	
22	Neg	Neg	Neg	Neg	Neg	**POS**	
23	Neg	Neg	Neg	Neg	Neg	Neg	
24	Neg	Neg	Neg	Neg	Neg	Neg	

Property 2: Lake Boga WP053 S35.51368 E143.68335 (Property 4 – 2009)
Zucchini Congo: < 5% incidence of moderate symptoms, 100 random samples collected, low weed incidence, no paddy melons

ELISA Results							
Virus	WMV2	CMV	PRSV	ZYMV	SqMV	WMV new	
Quedo 1	Neg	Neg	Neg	Neg	Neg	Neg	
2	Neg	Neg	Neg	Neg	Neg	Neg	
3	Neg	Neg	Neg	Neg	Neg	Neg	
4	Neg	Neg	Neg	Neg	Neg	Neg	
5	Neg	Neg	Neg	Neg	Neg	Neg	
6	Neg	Neg	Neg	Neg	Neg	Neg	
7	Neg	Neg	Neg	Neg	Neg	Neg	
8	Neg	Neg	Neg	Neg	Neg	**POS**	
9	Neg	Neg	Neg	Neg	Neg	Neg	
10	Neg	Neg	Neg	Neg	Neg	**POS**	

Property 3: Swan Hill WP055 S35.27983 E143.45529 (Property 5 – 2008, 2009)
Zucchini Houdini – 0% incidence, 200 random samples collected

ELISA Results							
Virus	WMV2	CMV	PRSV	ZYMV	SqMV	WMV new	
Milano 1	Neg	Neg	Neg	Neg	Neg	Neg	
2	Neg	Neg	Neg	Neg	Neg	Neg	
3	Neg	Neg	Neg	Neg	Neg	Neg	
4	Neg	Neg	Neg	Neg	Neg	**POS**	
5	Neg	Neg	Neg	Neg	Neg	Neg	
6	Neg	Neg	Neg	Neg	Neg	Neg	
7	Neg	Neg	Neg	Neg	Neg	Neg	
8	Neg	Neg	Neg	Neg	Neg	Neg	
9	Neg	Neg	Neg	Neg	Neg	Neg	
10	Neg	Neg	Neg	Neg	Neg	Neg	
11	Neg	Neg	Neg	Neg	Neg	Neg	
12	Neg	Neg	Neg	Neg	Neg	Neg	
13	Neg	Neg	Neg	Neg	Neg	Neg	
14	Neg	Neg	Neg	Neg	Neg	Neg	
15	Neg	Neg	Neg	Neg	Neg	Neg	
16	Neg	Neg	Neg	Neg	Neg	Neg	
17	Neg	Neg	Neg	Neg	Neg	Neg	
18	Neg	Neg	Neg	Neg	Neg	Neg	
19	Neg	Neg	Neg	Neg	Neg	Neg	

Property 4: Indian grower at Lake Boga/Tresco
Zucchini congo

- symptomatic individuals collected
- fennel weed sample collected (adjacent to virus infected zucchini!!!)

Appendix 4: The effects of climate change on plant virus epidemics of vegetables in Australia

Kyla Finlay, Jo Luck, Denis Persley and Brendan Rodoni.

Introduction

The potential impacts of climate change on plant virus epidemics in food crops has been little studied (Jones 2009) despite a review reporting that viruses are the predominant pathogen causing emerging infectious plant diseases (Anderson *et al.* 2004). Given the Australian vegetable industry was estimated to be worth approximately $3.1 billion dollars in 2006/07 (ABS 2008) this is a serious knowledge gap. The aim of this paper is to review potential impacts of climate change on vegetable viruses in Australia with a view to enhancing the capacity of the vegetable industry to implement integrated virus disease management programs and ultimately reduce the potential economic impact of climate change.

The Australian vegetable industry

Vegetable crops are grown in every Australian state. The gross value of produce was $3.1 billion in 2006/07 and Queensland consists of 32% of the total production while Victoria contributes 23% of the total gross value of vegetable commodities produced (Table 2). The gross value of total Australian agricultural production fell in 2006/07 by 6% primarily due to long standing drought conditions, however the value of vegetable crops actually increased by about 10% as the value of production of the top six vegetables (tomatoes, potatoes, lettuce, mushrooms, onions and carrots) substantially increased (ABS 2008). The top 6 vegetables include potato, tomato, lettuce, mushrooms, onions and carrots account for 55% of the total production (Table 1).

Table 1: Gross value of production of vegetable crops (2006-07)

Vegetable crop	Gross value	
	($m)	%
Potatoes	514.4	16.6
Tomatoes	296.0	9.5
Lettuces	282.9	9.1
Mushrooms	259.5	8.4
Onions	189.9	6.1
Carrots	158.4	5.1
Capsicums	138.6	4.5
Melons - watermelons	99.9	3.2
Cucumbers	91.6	3.0
Broccoli	87.6	2.8
Asian vegetables	77.0	2.5
Melons - rock and canteloupe	72.9	2.3
Beans, french and runner	72.8	2.3
Pumpkins	70.3	2.3
Zucchini and button squash	68.3	2.2
Cabbages	58.3	1.9
Sweet corn	53.8	1.7
Cauliflowers	52.7	1.7
Celery	42.3	1.4
Asparagus	26.2	0.8
Parsnips	20.4	0.7
Beetroot	14.0	0.5

Green peas	8.2	0.3
All other vegetables (not elsewhere included)	*347.2*	*11.2*
TOTAL	3103.0	100.00

Source: Australian Bureau of Statistics, Cat. 7503.0 (ABS 2008)

Table 2: Gross value of vegetable produce per state (2006-07)

State / Territory	**Gross Value ($m)**	**Gross value %**	**Top 4 crops in rank order**
Queensland	1002.3	32.3	Tomatoes, capsicum, Lettuce, potatoes
Victoria	704.4	22.7	Potatoes, Mushroom, lettuce, Tomatoes
South Australia	477.7	15.4	Potatoes, Onions, Carrots, Cucumber
New South Wales	428.9	13.9	Mushrooms, lettuce potatoes, rock melon and cantaloupe
Western Australia	267.8	8.6	Potatoes, carrots, Onions, Tomatoes
Tasmania	192.6	6.2	Potatoes, Onions, Carrots, Green peas
Northern Territory	29.3	0.9	Watermelons, Rock melon and cantaloupe, Asian vegetables, onions

Source: Australian Bureau of Statistics, Cat. 7503.0 (ABS 2008)

Climate change projections for Australia

Projected climate change trends for Australia (CSIRO & BoM 2007) are consistent with the latest IPPC projections for global climatic change (IPCC 2007). For Australia the best estimate for annual average temperature increases by 2030 are 1°C with slightly less (0.7° to 0.9°C) in the coastal south and north east and slightly more (1°C to 1.2°C) inland. (CSIRO & BoM 2007). This effect is projected to be lower in winter than in other seasons over the continent except for north-west Western Australia which is comparatively warmer in all seasons. By 2070 warming is more difficult to predict but best estimates of 1.8° – 3.4°C are projected depending on the emission scenario see (Nakićenović & Swart 2000). As before, less warming is expected in the south and north east coastal areas and more inland.

Associated with warming are changes in daily temperature extremes where an increased frequency of hot days and warm nights is projected. The average number of hot days (> 35°C) per year is expected to increase. There is also expected to be fewer cold days (< 0°C), with an accompanying decrease in frost frequency over the whole of Australia, particularly in the south over spring and summer.

By 2030, annual precipitation is expected to remain stable in the far north and decrease by 2-5% over most of southern and eastern Australia, particularly in winter and spring. By 2070, precipitation ranges are projected to be larger and more variable.
For example, the range of annual precipitation change for low to high emission scenarios, respectively, may change from -20% or -30% up to +10% or +20% in central, eastern and northern areas and between -30% to +5% in the south -10%. Decreases in the south-west in winter and spring could be as high as 30% or 40%.(CSIRO & BoM 2007).

Changes in relative humidity are predicted for most of Australia by 2030 with the small decreases (best estimate a 1% decline) in relative humidity projected over most of Australia. The largest decreases will be in the in the south and south-west in line with precipitation changes. By 2070 these decreases will be larger with declines of –ranging from 0.5 to 4 % over most of Australia. Again the largest decreases will be seen in the south and south–west. Comparatively larger decreases in winter and spring are also projected.

Changes in rainfall patterns are projected to result in greater frequency of unusual excesses or deficits. Extreme precipitation events (floods) are more likely in the north, compared to the south, but are nevertheless projected to be relatively widespread in summer and autumn. In addition, severe thunderstorms producing hail are likely to increase over the south-eastern coastal regions. In contrast, drought conditions are projected to increase over most of Australia with a higher likelihood predicted for the south-west. Accompanying this is a substantial increase in the risk of fire prone weather in south-eastern Australia.

Average wind speed is considered likely to increase in most coastal areas by a best estimate of 2-5% by 2030. The exceptions are around latitudes 30°S in winter (south-west Western Australia), and 40°S in summer (southern Victoria) where small deceases are likely. By 2070 wind speed increases are more difficult to predict but are likely to be of greater magnitude.

Climate change projections also indicate an increase in severe weather events. Tropical cyclones are likely to increase in intensity particularly off the north eastern coast of Queensland despite a possible decrease in the number of tropical cyclones overall.

Climate, plant growth and yield

Plant physiology and phenology is dependent on abiotic variables such as temperature and precipitation. The success of field crops, in particular, is closely tied to climatic and atmospheric variables. Consequently the expected change in the future temperature, atmospheric CO2 and rainfall patterns will result in important changes to agricultural and natural or wild ecosystems.

Temperature

The expected increase in minimum temperatures in the southern horticultural regions may be beneficial as they are likely to shorten the phenological cycle of field grown crops as shown in lettuce (*Lactuca sativa*).(Pearson *et al.* 1997) , French bean (*Phaseolus vulgaris*),(Wurr *et al.* 2000) and tomato (*Lycopersicon esculentum*) (Maltby

1995) and enable plants to start growing earlier in the season.(Fuhrer 2003). In such cases planting two crops per season may be possible. The projected reduction in frost risk may allow for solanaceous crops to be seeded *in situ* rather than transplanted from heated glasshouses as is currently the practice when frost risk is high (Peet & Wolfe 2000). High temperatures can cause premature bolting in lettuce. Parsley (*Petroselinum crispum*) silverbeet (*Beta vulgaris* ssp. *cicla*) and spinach (*Spinacea oleracea*).(Dioguardi 1995), lettuce tipburn (Dioguardi 1995), poor germination and heat stress in tomato plants (Riveroa *et al.* 2003), and reduce the curd quality of cauliflower (*Brassica oleracea*) (Oleson & Grevsen 1993).

CO_2

It is well established that elevated atmospheric CO_2 stimulates photosynthesis leading to higher plant yields for a wide range of species (Kimball *et al.* 2002; Ainsworth & Long 2005; Ziska & Bunce 2007) although there is an acclimatory response during long term exposure to elevated CO_2 (Drake *et al.* 1997) which appears to be greater for grasses and shrubs compared with trees and legumes(Ainsworth & Long 2005). The response for vegetables is unknown. C3 plants, which include most species and all vegetable crops, benefit from more efficient photosynthesis under higher CO_2. As they are bred to maximise yield, they are well placed for allocating these resources to harvestable products (Jablonski *et al.* 2002).

Experiments involving elevated CO_2 have shown yield increases for numerous vegetable crops. For example, potatoes (*Solanum tuberosum*) grown in controlled environment chambers or glasshouses, field-based open topped chambers or with Free Air Carbon Dioxide Enrichment (FACE) technology have primarily shown positive yield responses due to elevated CO_2 in the order of between 3% to 54% depending on CO_2 concentration, experimental design, cultivar and agronomy practice. (see review by (Finnian *et al.* 2005) This yield stimulation usually resulted in an increase in the number of tubers.

The weight of marketable fruit of glasshouse grown tomatoes grown over a two year experiment was increased by 5, 11 and 15% for elevated CO_2 levels of 375, 450 and 525 vpm respectively (Slack *et al.* 1988). Similarly, tomatoes grown in the field under polyethylene tunnels enriched with between 700-1000ml L^{-1} yielded 32% more fruit (Hartz *et al.* 1991). Field beans grown in controlled temperature environments at 700µL L^{-1} produced 25% more flowers and more nectar in total than those grown at ambient CO_2 (Osbourne *et al.* 1997). Plant biomass of capsicum (*Capsicum annuum*) grown in controlled environment chambers increased by 22% at elevated CO_2 of 700 cm^3 m^{-3} compared with ambient CO_2 levels but only under conditions where water and nitrogen were non-limiting (Peñuelas *et al.* 1995a). The biomass increase was largely allocated to fruit production with flowering and fruiting occurring earlier than in the control experiments. Cucumber (*Cucumis sativus*) yield was increased by 37% inside glasshouses when CO_2 concentrations were maintained at artificially higher levels (close to ambient) to compensate for the drop in CO_2 experienced by plant uptake during the autumn winter and spring in the airtight environment (Kläring *et al.* 2007).

Experiments conducted on beetroot (*Beta vulgaris*), carrot (*Daucus carota*) and bulb onion (*Allium cepa*). in day-lit controlled-environment cabinets at five CO_2 concentrations ranging from 350-750pvm and five temperatures from 12-18°C were used to estimate the potential impact of future climates in the UK (Wurr *et al.* 1998). Fresh weight yield increases of 19%, 9% and 13% for beetroot, carrot and onion

respectively were obtained using 2025 climate forecasts compared to 1992. Respective yield increases of 32%, 13% and 21% were obtained for 2050.
(Wurr *et al.* 2000) used a similar experiment with to estimate the effect of increased temperature and atmospheric CO_2 on French beans in growth cabinets. Future climate scenarios in the UK indicated that by the 2020's yield increases would be up 39-84% with a reduction in the time to maturity of between 6-15 days. By the 2050's yield increases could be as high as 51- 118% with earlier maturing date of between 9 – 25 days.

Responses of crop growth to elevated CO_2 may be complicated by inter-relating factors. The simultaneous temperature increase, for example, has been shown to counteract some of the positive benefits of elevated CO_2 for potato (Miglietta *et al.* 2000) and lettuce (Pearson *et al.* 1997).

Plants grown under elevated CO_2 are able to reduce their water loss through transpiration by the partial closure of stomata thereby increasing overall water use efficiency (Drake *et al.* 1997). Improved water use efficiency has been shown to increase soil moisture and therefore has the potential to prolong photosynthetic activity during drought (Donnelly *et al.* $_2$001) although accelerated growth may produce a higher leaf area index and a higher water loss potential which
could partially offset or even exceed the positive effects of an increased water use efficiency (Osborne *et al.* 2000). Nevertheless, substantial water use efficiency has been demonstrated on potatoes grown over a two year period in the Italian FACE (Magliulo *et al.* 2003). This improved water use efficiency may benefit crops grown in southern temperate regions of Australia where declines in rainfall will reduce water available for irrigation.

Climate and viruses

Plant virus epidemics are more likely when plants are exposed to environments where the viruses have not co-evolved with the wild ancestors of those plants so present a new opportunity for adaptation and potentially a new virus disease problem (Jones 2009). Climate change is likely to exacerbate this process as increased temperature and altered rainfall patterns will change the ranges and distribution of crops, weeds and native vegetation. This will lead to changes in the encounter rate between new or endemic viruses, their insect vectors and susceptible host plants. More encounters could lead to an increase in virus epidemics. Australia may be particularly susceptible to such plant virus epidemics due to our large contingent of endemic flora and fauna.

It is known that viruses have different temperature optima for multiplication. Viruses adapted to warmer climates are likely to increase as world temperature increases and temperate crops expand their geographic range whereas temperate viruses may diminish (Jones 2009). Geographic distribution of flora and insect fauna is likely to expand polewards as the temperature rises. Warmer growing conditions may alter virus evolution rates, modify host resistance as well as alter the virus physiology and subsequently virus-host-vector interactions (Jones 2009).

The interaction of drought stress and viruses is unclear. In drought prone areas such as the southern region of Australia there may be a reduction in cropping areas and new

virus encounters with hosts may diminish (Jones 2009). Conversely, drought and water stress may also increase host susceptibility leading to higher rates of virus proliferation (Anderson *et al.* 2004; Jones 2009). In a study of the effects of drought on sugar beet (*Beta vulgaris*) growth in plants affected with *Beet yellows virus* no interaction between the two stresses was elucidated (Clover *et al.* 1999).

Extreme precipitation events which are prevalent in the tropics and subtropics (and in summer and autumn in the south) may lead to conditions suitable for fungal pathogen growth which may weaken the plant and make it more susceptible for virus attack.

Climate and vectors

Changes in vector populations are one of the key drivers causing emerging infectious virus epidemics in plant crops (Anderson *et al.* 2004). Temperature directly affects the rate of growth and development of insects and there are a number of studies investigating insect response to elevated temperature (Bale 2002). Climatic warming should allow temperate annual and multivotine species, including aphids, whiteflies and thrips, to develop faster, produce more generations per year and expand their geographical range to higher latitudes and altitudes (Bale 2002).

Optimum temperature for development of pest species range between 22 and 38°C (Taylor 1981) with upper lethal thermal thresholds of between 40-50°C (Chapman 1988) depending on species and life stage. Eggs and nymphs (non-motile life stages) may be more susceptible to extreme temperature than adults. Some insects can exhibit evaporative cooling at low humidities allowing longer survival at higher temperatures in the short term (Prange 1996).

Optimum relative humidity for the development of many insect species is in the range of 60-80% with development times increasing when relative humidity decreases. (Cammell & Knight 1992). It is not known if the small decreases in relative humidity predicted under changing climate conditions will significantly alter insect development times (?ref?). As higher decreases in relative humidity are expected in the south, and particularly the south west, the affect of these changes in insect vectors should be examined.

In terms of elevated CO_2 there is relatively little experimental evidence of a direct effect on insects (Bale 2002; Stiling & Cornelissen 2007). Some insects such as the cotton bollworm (*Helicoverpa armigera*), prickly pearmoth (*Cactoblastis cactorum*) and hawkmonth (*Manduca sexta*) have been shown to detect changes in CO2 concentration, possibly as a mechanism for determining suitability of plant as a food source (Stange 1992; Stange *et al.* 1995; Abrell *et al.* 2005). The western corn rootworm beetle *Diabrotica virgifera* can detect changes in soil CO_2 concentration to locate corn roots (Bernklau & Bjostad 1998). It is more likely that the impact of elevated CO_2 on insect herbivores will be seen through the effect of physiological changes of CO_2 on the host plant.

Changes to plant physiology due to high CO_2 can increase the C:N ratio and alter concentrations of non-structural carbohydrates, starch and fibre content and other plant chemicals (Stiling & Cornelissen 2007). In general herbivores respond to plants grown under elevated CO_2 by "compensatory feeding", that is increasing food consumption

rates to compensate for lower food quality. This has been seen primarily in chewing larvae such as moths and butterflies (Osbrink *et al.* 1987; Lindroth *et al.* 1997; Agrell *et al.* 2000), beetles (Veteli *et al.* 2002), sawfly (Williams *et al.* 1997) and grasshoppers (Johnson & Lincoln 1990). Low nutritional quality of plant material can decrease insect growth rates and extend development times (Goverde & Erhardt 2003; Coll & Hughes 2008). In turn, this may lead to increases in mortality rates by extending the exposure time to natural enemies (or higher proportions of pathogenic bacteria and viruses) through feeding which could ultimately lead to reduced herbivore abundance (Kopper & Lindroth 2003).Species with the ability to adapt to low quality food may adjust more readily to the climate change (Bale 2002). The effects, however are highly specific to each insect-plant system and between insect groups (Coviella & Trumble 1999).

Whole-cell-feeders such as thrips show increased population sizes in response to high CO_2 (Bezemer & Jones 1998). Phloem feeding insects, which include aphids and whiteflies, have mixed responses to CO_2 enriched food. Aphids have been shown to improve their performance by increases in population size and decreases in development time (Awmack *et al.* 1997; Bezemer *et al.* 1998; Whittaker 1999) while others have observed decreases in aphid populations (Docherty *et al.* 1997). Yet others have observed no significant changes in population levels in aphids (Newman *et al.* 1999; Awmack *et al.* 2004) and whiteflies (Butler *et al.* 1986).

Preliminary results on the impact of elevated CO_2 on the physiology and feeding behaviour of the bird cherry oat aphid, *Rhopalosiphum padi,* on wheat from growth chambers experiments indicate no apparent differences in *R. padi* development under ambient CO_2 elevated compared eCO_2 (650 ppm) but a reduction in fecundity is indicated for nymphs grown under eCO_2. Indications are that *R. padi* engaged in less probing activity but ingested more from wheat grown under eCO_2 which could lead to a lower incidence of virus transmission (*P. Trębicki,* unpublished).

Extreme weather phenomenon will be the other major factor impacting on insect populations either directly through mortality or indirectly by shifting the occurrence and incidence of pest outbreaks. Heavy rainfall increases mortality of leaf dwelling insects by knock-down or flooding and has also been shown to deter oviposition (eg European corn borer, (Davidson & Lyon 1987). Prolonged wet conditions can also produce conditions favourable for diseases which can increase insect mortality. Similarly, hot dry conditions which are more likely in south-east and south-west Australia have been shown to reduce populations (eg aphids, (Garrett & McLean 1983). However, as drought can concentrate carbohydrates in the leaf, this may make the host plant more attractive for phytophagous insects (Mattson & Haack 1987).

Drought can favour plant sucking insects as drought stressed plants have a nutritional richer sap supply when water is scarse (Edwards and Wratten 1980). Aphids are more fecund on slightly wilted plants as opposed to well watered ones or severely wilted plants (Cammell & Knight 1992).

Case Studies

Development and perpetuation of an infectious plant disease involves interactions between the pathogen causing the disease, the vector that may carry or transmit the

pathogen, the plant species serving as a host for the pathogen and the present climatic environment (Fig 1). Any alteration in any one of these components can influence the interactions and ultimately the degree of disease severity. For example, changes in climate can affect vector growth and development which in turn is likely to impact on disease transmission rates which will affect plant health and disease development or severity.

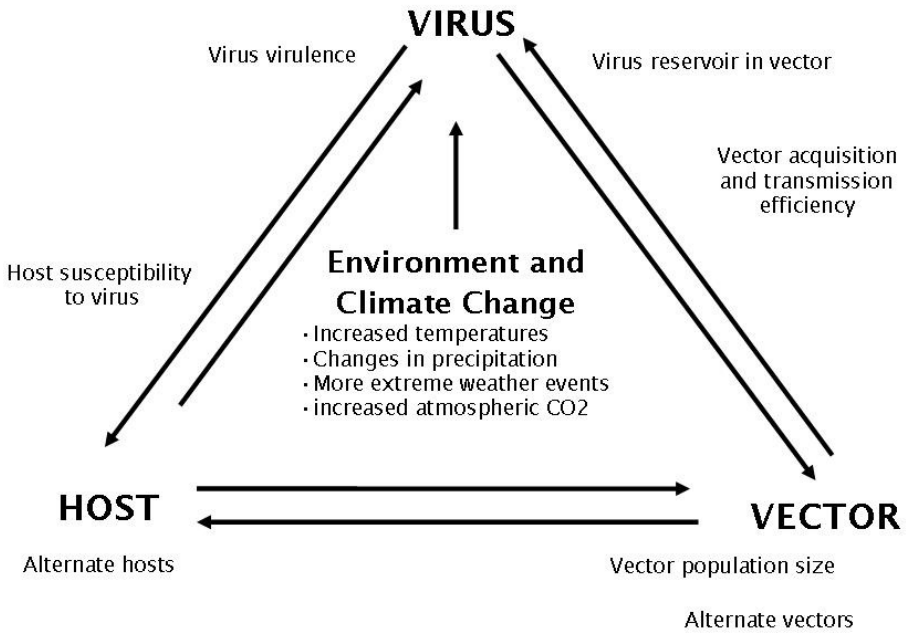

Figure 1: Elements of a plant virus pathosystem involves interactions between the virus causing the disease, the vector that may carry or transmit the virus, the plant species serving as a host for the pathogen and the present climatic environment. Any alteration in any one of these components can influence the degree of interaction and ultimately disease severity.

The diversity of crops and geographical extent of the industry makes it difficult to generalise on potential impacts of climate change. There is any number of different combinations of interactions that could potentially occur between host, vector, virus and the environment, including the possibility that some factors could reinforce or counteract the host, pathogen or insect vector. It is therefore difficult, and perhaps completely unfeasible, to construct generalisations about the final outcome that would apply to many different crops and viruses. The approach taken here is to examine three case studies that illustrate how climate change may ultimately affect specific virus-host-vector systems. By doing this we hope to increase awareness of the possible effects of climate change on viruses in vegetable crops and identify critical information required to develop effective management regimes. Ultimately the aim is to enhance the capacity of the vegetable industry to respond to possible climate change impacts.

The three case studies chosen for further examination are (1) *Tomato spotted wilt virus*, vectored by thrips; (2*) Tomato yellow leaf curl virus*, vectored by whiteflies and (3) *Watermelon mosaic virus*, vectored by aphids.

These case studies were selected on the basis of importance to industry and to encompass both a range of vegetable crops and insect vectors of plant viruses.

1. Tospoviruses

Tomato spotted wilt virus *(TSVW)*

[Family Bunyaviridae, genus Tospovirus]

Background - TSVW

A disease referred to "spotted wilt" was first described by (Brittlebank 1919) from tomato plants in Victoria, Australia. Within ten years it was known from all Australian states causing considerable losses to the tomato industry (Best 1968) and is now found throughout the world (Jones 2005). The causative agent was discovered to be a virus hence the name *Tomato spotted wilt virus* (TSWV) (Samuel *et al.* 1930).

In Australia there were serious outbreaks throughout the twentieth century including a steady increase in disease incidence from the early 1990s with many vegetable crops in New South Wales, Victoria, South Australia, Queensland and Western Australia greatly affected (Clift & Tesoriero 2002)Severe epidemics have occurred in vegetable crops in Adelaide in 2000 (Anon 2000) and tomato, capsicum and lettuce crops in Perth 2000-01 (Coutts & Jones 2002a)

TSWV is the most widespread of four Tospoviruses found in Australia (Persley *et al.* 2006). Worldwide its current host range is over 1000 species with the families Solanaceae and Asteraceae prominently represented (Persley *et al.* 2006). New hosts are recorded regularly (Parrella *et al.* 2003). Vegetable crops affected include artichoke, bell pepper, broad bean, common bean, cabbage, capsicum, cauliflower, celery, chicory, chilli, cucumber, eggplant, endive, lettuce, pea, potato and spinach, sweet pepper and tomato with lettuce, tomato, capsicum and potato considered the most severely affected in Australia (Persley *et al.* 2006)

Symptoms of TSWV include mottling, distorted fruit, chlorosis, ringspots, necrotic spots, reduced yield and vigour, streaks and stunting and sometimes plant death (Persley *et al.* 2006) However the symptoms are also highly variable from host to host and for any given host plant depending on its age or environmental conditions (Best 1968)

Vectors - Thrips (Thysanoptera)

Established vectors of TSWV in Australia include *Frankliniella occidentalis*, (western flower thrips), *F. schultzei* (tomato thrips), *Thrips palmi* (melon thrips) and *T. tabaci* (onion thrips). *Scirothrips dorsalis* (Oriental tea thrips, chilli thrips) has been reported

as a vector of TSVW previously, albeit requiring further confirmation (Mound 1996). It is not listed as such in a recent review of tospovirus epidemics (Pappu *et al.* 2009)

Frankliniella occidentalis – *Western Flower Thrips*

The western flower thrips (WFT) is considered the most efficient vector of TSWV (Wijkamp *et al.* 1995) due to its high fecundity and polyphagy (German *et al.* 1992). Certainly increased losses from TSWV infection co-incided with the incursion and wide dispersal of *F. occidentalis* in 1993 in Australia (Malipatil *et al.* 1993).

WFT is a successful invasive pest partly because it is extremely polyphagous with a wide host range of over 200 plant species (Jones 2005). This includes the following vegetable crops: bean, beetroot, cabbage, capsicum, carrot, chilli, courgette, cucumber, eggplant, lettuce, onions, ornamental gourd, pepper, parsley, pea, pumpkin, squash, sugarbeet, sweet pea, tomato, wild radish and zucchini.

The WFT has six developmental stages: (1) egg, (2) larval instar one, (3) larval instar 2, (4) pre-pupa, (5) pupa and (6) adult. (Fig. 2) The two larval stages feed on leaves, buds and flowers and at the base of some veg fruits (EPPO 2002)

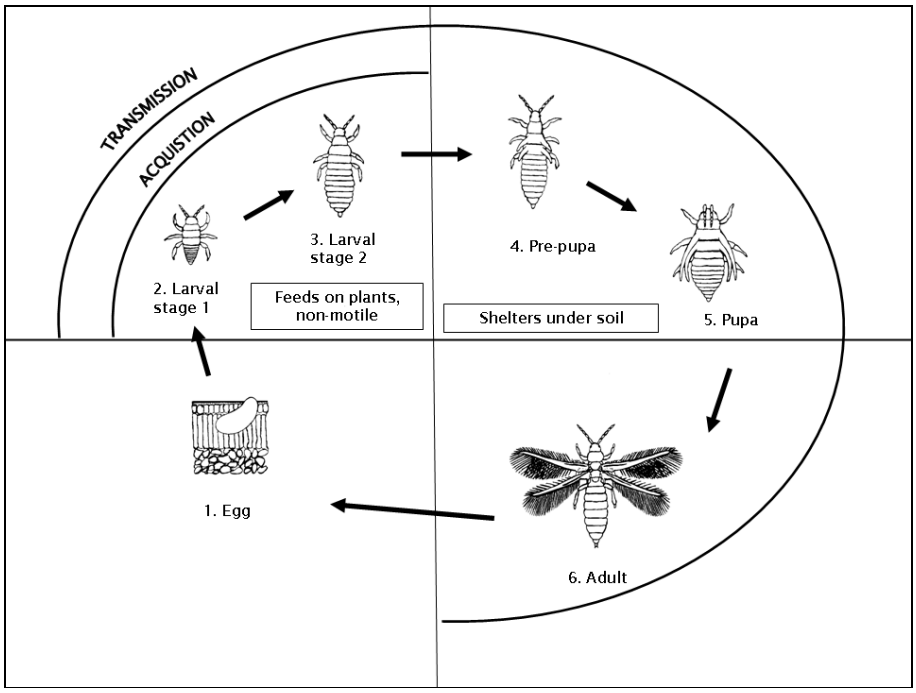

Fig. 2. Life cycle of western flower thrips (*Frankliniella occidentalis*) showing the stages where TSVW acquisition and transmission occurs.

Development of *F. occidentalis* takes between 9-39 days from egg to adult depending on temperature (Reitz 2008). The most favourable temperature for development is around 25-30°C see refs in (Reitz 2009). Females only require a short pre-ovideposition period of a few days after emergence (Reitz *et al.* 2008) before they are able to ovideposit which they can continue to do throughout their life time producing between 150-300 eggs (Hill 1994). The life cycle is mostly continuous: all stages of thrips can be found all year round (Hill 1994).

Acquisition of the virus by thrips can only occur during the first and second larval feeding stages following feeding on infected plants (Figure 2) (Reitz 2009) Plants that can be infected by TSWV and which can be colonised by a thrips species that can acquire TSWV if the host plant is infected, are refereed to as "reservoir hosts" as these plant species are critical for the development of a spotted wilt disease epidemic. The majority of thrips become viruliferous in the second larvae stage (Wijkamp & Peters 1993). A minimum acquisition period of 15-30 mins is required (Sakimura 1962) but acquisition ability decreases with age (van de Wetering *et al.* 1999). Once acquired the virus is persistently passed through each insect stage from larvae to adult stages (Sherwood *et al.* 2000b) and thrips remain infective for life (Sakimura 1962). Adult longevity is significantly reduced by Tospovirus infection (German *et al.* 1992)

Between acquisition and transmission there is a latent period where the thrips are not infective (Wijkamp & Peters 1993) but in which the virus multiplies (German *et al.* 1992). The latent period is temperature dependent, occurring faster at relatively higher temperatures. For example 7.1 days are required for 50% of the larvae that eventually transmit to begin transmitting at 20°C and only 3.5 days at 27°C. (Wijkamp & Peters 1993)

Transmission can occur rapidly with as little as 5 mins feeding (Coutts *et al.* 2004). Second stage larvae are more efficient at transmitting the disease than adults (Wijkamp *et al.* 1993) which has important implications for the spread of the disease as second instar larvae are non flying and do not readily move from plant to plant (Reitz 2009). There is, however, variation in TSVW transmission efficiencies between different WFT populations (van de Wetering *et al.* 1999), also between different regions and also between populations collected from the same region which makes prediction of virus spread more difficult.

Other thrips vectors of TSWV

T. tabaci and *F. schultzei* are both present throughout Australia: *T. tabaci* from all states and *F. schultzei* from all mainland states (AICN 2008). Both species have been implicated in significant outbreaks of TSVW in vegetable crops in the eastern States in the 1994/95 season (Clift & Tesoriero 2002). The most seriously affected crops include pepper, lettuce, tomato and potato (Pappu *et al.* 2009)

Thrips palmi is a polyphagous pest of Curcurbitaceae and Solanaceae in south east Asia, Japan, Florida, the Caribbean and Australasia (QDPI&F 2005). It has been detected only in Northern Australia (Young & Zhang 1998; QDPI&F 2005), particularly in south east Queensland where it is well established and is associated with cucumbers, melons, capsicum, zucchini, pumpkin, beans and squash in the Northern Territory (Layland 1991). *T. palmi* has been recorded but not well established in north Queensland.

All species of the family Thripidae, to which these vectors belong, have similar life cycles to western flower thrips with eggs inserted into plant tissue, two larval stages and two pupal stages. Life cycles commonly take around 20 days in warm conditions but certainly less is known about these alternate vectors compared to western flower thrips (Mound 1996).

Virus accumulation and transmission varies between the vectors of TSVW with the two species of *Frankliniella* being more efficient than the two species of *Thrips* possibly attributed to the higher accumulation of virus in the adults of the former genus (Inoue *et al.* 2002). Interestingly, there have also been changes in relationships between tospoviruses and vectors over time. *T. tabaci*, for example, no longer transmits some current isolates of TSWV (Nagata *et al.* 2004)

Potential climate change effects

Increases in ambient temperatures will hasten the development rate of the thrips leading to more generations per year and an increase in population levels. In general there is likely to be a shift in populations southwards as Northern Australia temperatures become too hot although each species will have different thermal thresholds. Thermal models do indicate that *T. tabaci* has a capacity to adapt to local climate conditions (Bergant *et al.* 2005)

In field trials on lettuce and pepper in Western Australia *T. tabaci* populations predominanted earlier in the season when the climate was cooler, while *F. schultzei* occurred later (Coutts *et al.* 2004)

However, the thigmotactic behaviour of thrips larvae and adults that makes detection difficult (Reitz 2009) may protect them from the heat. Similarly, as eggs are laid in plant tissue and the pupal stages (prepupa and pupa) shelter under soil or in the bases of leaves they are somewhat protected from excessive heat.

Higher population levels will cause more damage to crops as excessive feeding by thrips can cause flower and fruitlet distortion as well as abortion (Hill 1994). Damage to fruit also occurs via female oviposition as the thrips eggs are inserted into the plant epidermis using a serrated ovidepistor {Reitz, 2009 #688). The embedding of the eggs in leaf tissue, or plant parts such as bean pods, cause spotting on fruits and the downgrading of quality. These puncture wounds can potentially cause more entry wounds for pathogens.

Increasing temperatures predicted under climate change could have a significant effect on TSWV transmission efficiencies of the thrips. In laboratory studies second stage larvae of *F. occidentalis* has a decreased transmission efficiency at higher temperatures (52.8% at 20°C ; 32.9% at 27°C) while the adult transmission rate increased (2.3% at 20°C compared with 10.1% at 27°C) although the rate of adult transmission was significantly lower than the rate of second stage larval transmission at the same temperatures. (Wijkamp *et al.* 1993). The overall transmission rate for larvae and adults combined was actually reduced as the temperature increased (55.1% at 20°C to 43.0% at 27°C) (Wijkamp *et al.* 1993).

The effects of elevated CO_2 on the interaction between thrips and host plants has been little investigated. A number of studies have indicated no significant difference in numbers of WFT in ambient and elevated CO_2 (Butler 1985; Hughes & Bazzaz 1997; Heagle 2003) In a study of an infestation of WFT on greenhouse grown *Asclepias syriaca* (common milkweed) (Hughes & Bazzaz 1997) showed that, although there were no difference in numbers of thrips at either concentration of CO_2, the amount of

damaged leaf area per capita (consumption rate) of thrips was significantly greater at elevated CO_2 (700 µl 1^{-1}) compared to ambient CO_2 (350 µl 1^{-1}). However, the increased herbivory was more than compensated for by the increased growth rate of plants at elevated CO_2 with the net outcome being more advantageous to the plant as there was more undamaged leaf area available for photosynthesis. A similar result was found for WFT infesting *Trifolium repens* (white clover) at elevated (745 µl 1^{-1}) and ambient CO_2 (396 µl 1^{-1}) grown in greenhouse chambers.

Severe weather events predicted under climate change are also likely to impact on populations. Heavy rain washes thrips from surface of the leaves (Young & Zhang 1998) and monsoonal wet season rain has been shown to significantly reduced populations of *T. palmi* in the Northern Territory (Layland 1991). Similarly, the pupal stages of *T. palmi* are very sensitive to soil moisture and can be attacked by soil fungi and bacteria if the soil moisture is too high hence this species population diminishes in the Northern Territory wet season (Young & Zhang 1998).

Wind dispersal of adult thrips is aided by their large surface area to volume ratio and fringed wings (Sherwood *et al.* 2000b). *T. tabaci* and *F. schultzei* have been shown to migrate over long distances (Wilson & Bauer 1993). In contrast, *F. occidentalis* does not disperse as readily but tends to move with infested material (Clift & Tesoriero 2002) Projected increases in wind speed in coastal areas could be a factor in increasing thrips dispersal in general but may also aid in increasing the dispersal capacity of *F. occidentalis*.

Widely distributed weeds and other species can act as alternate hosts of TSVW between cropping seasons (Cho *et al.* 1986; Groves *et al.* 2002). Thrips alighting on these less than desirable hosts tend to probe for relatively short periods before moving on in search of a more suitable host plant. This persistent shallow probing may actually increase the virus load in unsuitable host species (German *et al.* 1992). Weeds and widely distributed naturalised hosts may play and evermore important role as reservoirs of virus under climate change as increasing temperatures and altered rainfall patterns may enhance their growth and distribution in some areas.

2. Geminiviruses

Tomato yellow leaf curl virus *(TYLCV) (Family Geminiviridae, genus Begomovirus)*

Background – TYLCV

Tomato yellow leaf curl virus (TYLCV) symptoms were first reported from Jordan Valley, Israel in tomatoes as early as 1929 (Cohen & Lapidot 2007). In 1959, similar disease symptoms and an accompanying outbreak of whiteflies caused the failure of the entire tomato crop in this area (Picó *et al.* 1996). Following this, the first description of the virus was published (Cohen & Harpaz 1964). TYLCV is one of the most devastating diseases of tomato (Picó *et al.* 1996) with yield losses of up to 100% in greenhouse tomato crops in Europe and Middle East and crop losses of up to 100% in many tropical and subtropical regions of the world (Picó *et al.* 1996; Czosnek & Laterrot 1997).

Symptoms of the virus appear 2-4 weeks after inoculation becoming fully developed after around 8 weeks. (Picó *et al.* 1996). Affected plants are stunted or dwarfed with leaves that are misshapen, thicker and bend downwards often showing chlorosis of the veins. Leaflets are rolled upwards or inwards and yellowing, however only new growth produced after infection is affected. Purpling can also occur on the lower surface of leaflets. Plants infected at early stages of development shed flowers and have high yield loss due to fruit drop and / or fewer and smaller fruits which can be dry and unmarketable (Thomas & Persley 2007)

TYLCV is now regarded as a virus disease complex found in Europe, sub-Saharan Africa, Asia, Australia, the Caribbean and the United States (OEPP/EPPO 2005). The arrival of TYLCV in Australia is relatively recent with an outbreak of the virus in cherry tomato crops near Brisbane in 2006 (ProMED & mail 2006). By 2009 the virus had spread throughout the $AUD 70 million tomato industry in Bundaberg (Queensland) with more than 80 % of crops infected (Van Brunschot et al., 2010)

TYLCV develops in only a few species of several families, primarily affect members of Solanaceae (Picó *et al.* 1996). Tomato (*Lycosperisicon esculentum* Mill) is the major host of TYLCV (Thomas & Persley 2007) with other vegetable hosts including Capsicum (*Capsicum annuum* L.) chillies (*C. chinense* Jacq.) and beans (*Phaseolus vulgaris* L.) (Tesoriero & Azzopardi 2006; Thomas & Persley 2007).

Vector - *Bemisia tabaci* Biotype B (Gennadius) (Aleyrodidae: Hemiptera)

The silverleaf whitefly (SLW, *B. tabaci* biotype B) is considered to be one of the most important pests worldwide. Also variously called the tobacco, cotton or sweet potato whitefly it is a major crop pest in tropical and subtropical regions (Jui *et al.* 2007) but can survive in some mild temperate climate providing overwintering hosts exist (De Barro 1995)The SLW colonises and feeds on many plant species, including ornamental plants, vegetables, cotton and weeds. Damage caused by SLW is through direct feeding on the sap of colonised plants and the rapid development of large SLW populations; this can cause physiological damage such as the “silverleaf” condition in some cucurbits and uneven ripening in tomatoes. SLW infestations can also lead to contamination of crops by honeydew and sooty mould, resulting in low quality products.

Populations of *B. tabaci* are designated as biotypes based on host range, fecundity, developmental rates and the ability to transmit viruses (see (De Barro 1995). Biotype B has a greater host range than other biotypes with over 600 species (Picó *et al.* 1996)). It also has strong pesticide resistance and a higher fecundity which has increased the transfer of viruses between cultivated and weed hosts and facilitated new plant virus interactions (Seal *et al.* 2006)

B. tabaci biotype B was first detected in Australia in Darwin in 1994 but was considered present in Australia for at least one year prior (De Barro 1995; Gunning *et al.* 1995)It is now present in most mainland states of Australia and causes significant damage to numerous crops including curcurbits, eggplants, beans, sweet potatoes and tomatoes (Stonor *et al.* 2003).

The general life cycle of *B. tabaci* biotype B has six phases: (1) egg, (2) first instar nymph, (3) second instar nymph, (4) third instar nymph, (5) fourth instar nymph or red-eye pupae and (6) adult (Fig 3). (QDPI&F 2006). The first instar crawl a short distance before finding a sap source, whereas the second, third and fourth instars are stationary (Siva-Subramaniam *et al.* 2007). Adults develop in the pupal case before emerging, leaving visible exuviae on the leaf surface.

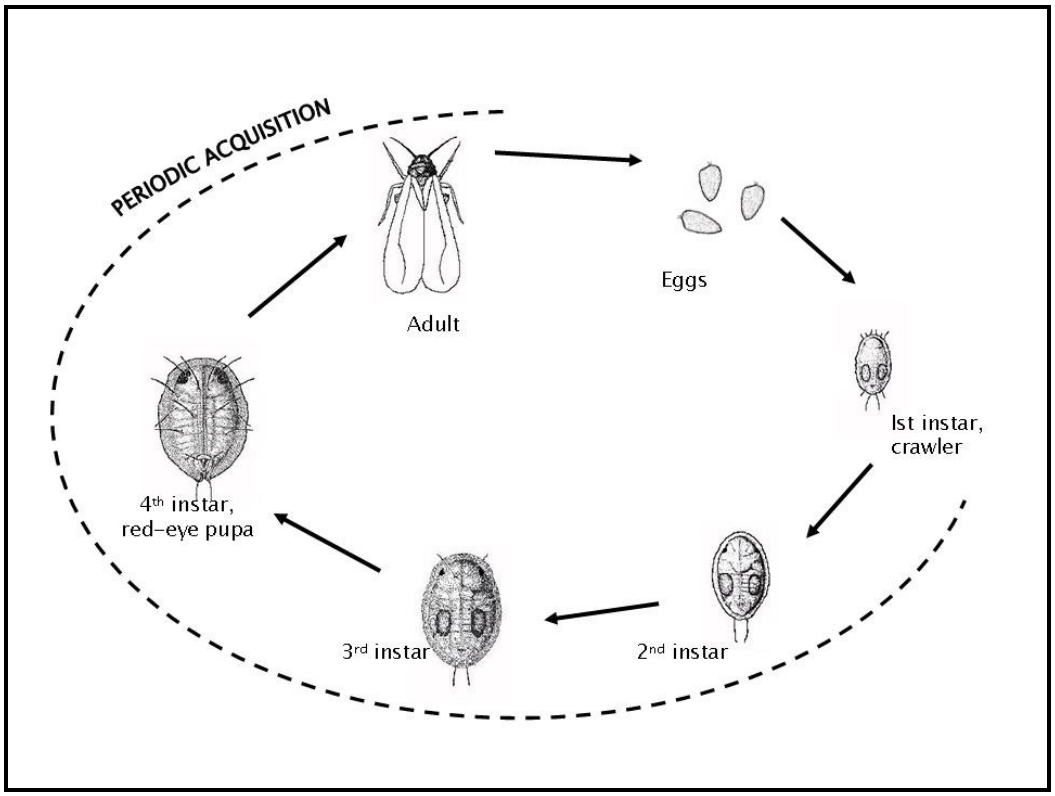

Fig 3: Life cycle of silverleaf whitefly, *Bemisia tabaci*

The length of the life cycle depends on temperature taking only 18-28 days in warm weather and 30-48 days in winter (Siva-Subramaniam *et al.* 2007) and producing 11-15 generations per year (De Barro 1995). Females can lay between 50-400 eggs averaging around 160 (Siva-Subramaniam *et al.* 2007) Life expectancy of viruliferous insect populations are around 20% lower than non infected insects and long term association of the virus in the vector correlated with lower fecundity (Rubinstein & Czosnek 1997).

The minimum virus acquisition period ranges from 15-60 mins (Czosnek 2007; Thomas & Persley 2007) Once acquired there is a latent period in which the vector is unable to transmit the virus which ranges from 17 to 24 h (Picó *et al.* 1996) (Tesoriero & Azzopardi 2006) after which the virus can be passed onto healthy plants within 15 mins of feeding.(Tesoriero & Azzopardi 2006). The vector is infective for an average of 10-12 days (Cohen & Harpaz 1964) but is unable to re-acquire the virus during that time. This phenomenon is called "periodic acquisition" and refers to the inability of the vector to re-acquire a virus from a host plant while it is still able to transmit virus particles (Cohen & Harpaz 1964). Only a single insect is required to infect a host plant following the 24 hour acquisition/latency period (Czosnek 2007). Nymphs and adult

whiteflies are equally as effective at acquiring the virus (Picó *et al.* 1996; Moriones & Navas-Castillo 2000). TYLCV spread from plant to plant occurs via the active adults (Thomas & Persley 2007).

Transmission efficiency increases as the duration of the feeding time increases (Thomas & Persley 2007) with females are times more efficient at virus transmission (Czosnek 2007). TYLCV transmission can persist for the whole life span of the insect although efficiency declines with age (Rubinstein & Czosnek 1997)

Potential climate change effects

Climatic changes that positively or negatively affect the survival, fecundity and development of *B tabaci* biotype B will, in turn, impact on the level of virus transmission in host plants.

Hot dry climates with irrigated crops provide a very suitable climate for the insect vector (Gunning *et al.* 1995) and with shorter generation times, large populations can be built up over the summer months. The optimal temperature for development is considered to be around 25-28°C (Wagner 1993) Oviposition and survival rate of the immature stages can be impaired by extreme temperatures (Gerling 1986). (Picó *et al.* 1996) reports that temperatures below 9°C and above 40°C are lethal however, although pre-ovipositional adults have been shown to survive temps of -2°C for 80 hours These thermal thresholds would therefore not be beyond the ability of the insect to survive any increase in temperatures due to climate change and may lead to build ups of large populations, although consecutive days of high temperatures as predicted under climate change, may be detrimental to whitefly survival.

Begomoviruses, in general, seem to adapt to new hosts readily with frequent reports of new strains infecting previously unaffected hosts (Seal *et al.* 2006). Warmer conditions can potentially increase virus evolution rates which may lead to more virulent strains with broader host ranges, better transmission efficiencies or a higher reservoir of virus particles in the host (Picó *et al.* 1996) and ultimately a more severe epidemic.

The predicted increase in severe weather events projected under climate change will impact on the population of *B. tabaci* variously. Oviposition can be impaired by rain, {Cohen, 1990 #766} and populations decrease after heavy rain showers (Varma & Malathi 2003; Cerkauskas 2004) A modelling study suggested that the fourth nymphal stage was particularly prone to dislodgment by wind, rain and chewing predators with windspeed was considered the most important factor for dislodgement of all life stages (Naranjo & Ellsworth 2005). Climate change projections of stronger winds and more intense cyclone activity in the north may facilitate a faster and greater dispersal of *B. tabaci* to new areas as long distance migration can occur via wind currents (Lenteren & Noldus 1990; De Barro 1995). Drought could also increase mortality and disrupt development and limit its population size and distribution in susceptible areas. Water stress to host plants may increase host susceptibility leading to higher rates of virus proliferation (Anderson *et al.* 2004; Jones 2009)

The survival rate of the immature stages of SLW is adversely affected by extreme high and low relative humidity (Horowitz *et al.* 1984) (Gerling 1986).Optimum relative humidity for *B. tabaci* insect development is between 30-60% (Picó *et al.* 1996) and

decreases in survival of adults and impairment of oviposition have been recorded at relative humidities below this (Gerling 1986; Cohen 1990). In Australia higher relative humidities occur on the northern and southern coastlines ranging between 60-80% depending on the time of year (BoM 2009)

An important factor in the survival of SLW is the continuous availability of suitable hosts, which may be cultivated or weed species. Higher temperatures and changing rainfall patterns are likely to alter the distribution of TYLCV hosts (Jones 2009) which will either increase or decrease in the likelihood of the virus encountering new host plants. For example, SLW survival is limited in some cotton growing regions of Australia due to the rotation of cotton with other non-host crops such as cereals or legumes and a lack of host weed species in winter. A predicted increase in winter temperatures may prolong the survival of weed hosts through winter (known as a "green bridge") increasing the insect's ability to overwinter. Conversely, under the wet and warm climatic conditions of the tropical north *B. tabaci* populations may be reduced via infection by fungal pathogens (Faria & Wraight 2001), a situation which is only likely to improve as the temperature and humidity increases.

3. Potyviruses

Watermelon Mosaic Virus

[Family Potyviridae, genus Potyvirus]

Background

Watermelon mosaic virus (WMV), formerly known as WMV2 is widely distributed throughout the world (ICTVdBManagement 2006; Gibbs *et al.* 2008) mostly in temperate and Mediterranean regions (Desbiez & Lecoq 2004)

It was first proposed as separate virus by (Webb & Scott 1965) who studied the WMV complex and divided the isolates into two groups based on cross-protection inoculations, serology and host range differences. WMV1 has since been renamed Papaya ringspot virus (PRSV) reverting WMV2 back to WMV.

WMV has a wide experimental host range, infecting 178 species in 79 genera and 27 families Edwardson and Christie 1991 including Leguminosae, Chenopodiaceae and Euphorbiaceae (Webb & Scott 1965). The virus causes major disease problems cantaloupe, cucumber, pumpkin, squash, and watermelon and reduces fruit production and quality in squash and other cucurbits, (Purcifull *et al.* 1984) Alternative weed hosts and abandoned and volunteer cucurbit species can act as reservoirs for infection especially between cropping seasons. (Desbiez & Lecoq 2004) (Coutts 2006) In Queensland and elsewhere, wild and native curcurbits such as *Cucumis myriocarpus* (prickly paddy melons), *Cucumis anguria* (wild gherkins) and *Citrullus lanatus* (pie or jam melons) can act as reservoirs for the virus but are often symptomless (Horlock & Persley 2004).

Symptoms of WMV are generally less severe than PRSV-W or *Zucchini yellow mosaic virus* (ZYMV). Symptoms of WMV include mosaic, mottle, leaf distortion, plant stunting and vein banding (Horlock & Persley 2004; ICTVdBManagement 2006). Despite this, fruit ripen normally and are of good quality {Horlock, 2004)

Virus distribution in Australia

WMV has been found in Australia in most districts were commercial crops of cucurbits are grown including Queensland {Greber, 1978 #884}(Horlock & Persley 2004), Western Australia (Coutts & Jones 2005; Coutts 2006), NSW (Jelinek 2009), Victoria and Northern Territory (Coutts & Jones 2005). In Western Australia WMV has been found affecting cucurbits in Perth, Kununurra Carnarvon and Broome (Coutts 2006)

Vectors

WMV can be transmitted by 38 species of aphids in 20 genera experimentally and in nature is transmitted predominantly by three species: the potato peach or green peach aphid (*Myzus persicae* (Sulzer)), the cowpea aphid (*Aphis craccivora* Koch) and the cotton or melon aphid (*Aphis gossypi* Glover) (Aphididae: Hemiptera) (Shukla *et al.* 1994).

All three species are found worldwide including throughout Australia (Blackman & Eastop 2000; AICN 2008) In Australia the life cycle of *M. persicae* generally alternates between sexual reproduction on the primary host (*Prunus persica* and other *Prunus* species) where eggs are laid. Females hatch in spring and their descendants disperse to secondary host plants where they produce many pathenogenetic generations (Blackman & Eastop 2000). *Aphis craccivora* is completely parthenogenetic and *A. gossypi* can be either which may be due to issues with the taxonomy of the species (Blackman & Eastop 2000). All species are extremely polyphagous.

Potyviruses are transmitted in a noncirculative non-persistent manner (Horlock & Persley 2004; Ng & Perry 2004) characterised by short acquisition and inoculation times. For *M. persicae* acquisition periods can be as short as 10-60 seconds (Purcifull *et al.* 1984) with retention capacity and capability to transmit the virus for several hours following acquisition (Horlock & Persley 2004). Due to these very efficient transmission capabilities crop infections can be severe without accompanying large populations (Horlock & Persley 2004). Transient winged aphids often play a major role in WMV transmission as they search for food sources but do not colonise plants. Transmission may not be efficient in some of these aphid species but many aphids probe many plants and acquire and transmit WMV as they do so. *Myzus persicae* transmitted WMV more frequently than all other aphids tested in laboratory transmission studies on muskmelon (Castle *et al.* 1992). Field collected *M. persicae* transmitted at higher rates than laboratory counterparts (Castle *et al.* 1992)

Nymphs and adults are equally capable of acquiring and transmitting the virus efficiently (Namba & Sylvester 1981) although adults are responsible for virus spread through mobility and ability to be carried long distances by wind (Horlock & Persley 2004)

Virus infections are more damaging when the crop is infected early before flowering. For example, infections before flowering caused a 50% loss of production in melons in

Spain whereas infection between flowering and fruit set resulted in a loss of only 26% (Alonso-Prados *et al.* 1997)

Potential climate change effects

The predicted increase in temperature due to climate change over the next 20 – 50 years is likely to increase the development rate of aphids, their mobility within a crop and potentially aid their long distance dispersal (Canto et al., 2009). In controlled temperature chambers, Berg (1984) found that the reproductive period of *A. craccivora* began earlier and was shorter at higher temperatures up until 30oC and the upper thermal threshold of this aphid species was 35oC. He also found that the growth potential of the aphid was dependent on the host species with higher growth rates observed on *Vicia faba* than on a number of weed species (Berg 1984).

Mild winters provide favourable conditions for growth of virus source plants and aphid vector food plants (Nelson & Tuttle 1969) and this also helps to sustain larger populations of overwintering aphids (Bale et al. 2002). Thermal thresholds for dispersing aphid species will also be reached earlier in the year as seen in aphids flight patterns (Fleming & Tatchell 1995) which can lead to early migration.

As stated previously there has been an ambiguous response of aphids to elevated CO_2 ranging from decreases in performance (Thompson *et al.* 1993; Bezemer *et al.* 1999), no change (Docherty *et al.* 1997) to increases in performance (Awmack *et al.* 1996; Bezemer & Jones 1998; Bezemer *et al.* 1999). For example, the daily rate of production of nymphs for the potato aphid *Aulacorthum solani* were higher on broad bean (*Vicia faba*) grown in elevated CO_2 compared with ambient, but development time was not affected. On tansy (*Tanacetum vulgare*), however, development time was shorter but with no impact on the production of nymphs (Awmack *et al.* 1997).

Discussion

For Australia the best estimate for annual average temperature increases by 2030 are 1°C with slightly less (0.7° to 0.9°C) in the coastal south and north east and slightly more (1°C to 1.2°C) inland. (CSIRO & BoM 2007). It is predicted that these changes will result in an increase in daily temperature extremes, more extreme weather events and a reduction in rainfall in southern and eastern Australia. Plant physiology is expected to change and the expected increase in minimum temperatures in the southern Australia is likely to shorten the phenological cycle of field grown crops and enable plants to start growing earlier in the season. Increased CO2 will increase photosynthetic rates and this may alter the feeding habits of key insect vectors and their ability to acquire and transmit plant viruses.

It is difficult to predict how these changes will affect plant virus epidemics in vegetable crops. Plant virus epidemics are more likely when plants are exposed to environments where the viruses have not co-evolved with the wild ancestors of those plants (Jones 2009) and climate change is likely to promote this phenomenon as changes in temperature, rainfall and frost frequency will alter the current geographic range of crops,

weeds and insect vectors. Extreme weather events (cyclones, floods) may also increase the dispersal capacity plant and insect species.

The three host/virus/vector scenarios discussed in this paper highlight how difficult it will be to predict the impact of a changing environment on plant virus disease epidemics in vegetables. It is possible for example that existing resistant cultivars could be affected as some R genes fail or are less effective under high temperatures (e.g. above mid 30s). Another possibility is that crops, particularly irrigated vegetable crops, could be more vulnerable to virus infection as they are the green islands in a dry landscape attracting migrating insect populations. The extended production cycles of many vegetable crops that will result in earlier plantings because it is warmer and there are less frosts, will reduce the length of production breaks and therefore enhance the green bridge for viruses and their insect vectors.

It is clear that cropping systems, insect vector incidence and the abundance of alternate hosts for viruses and insect vectors will change with climate change. To counteract increased losses in vegetable production it is imperative that each cropping system be carefully monitored for the emergence of new plant disease epidemics.

References

Abrell L, Guerrenstein PG, Mechaber WL, Stange G, Christensen TA, Nakanishi K, Hildebrand JG (2005) Effect of elevated atmospheric CO2 on oviposition behaviour in *Manduca sexta* moths. *Global Change Biology,* **11**, 1272-1282.

ABS (2008) Value of Agricultural Products Produced, Australia, 2006-07. *Cat 7503.0.*

Agrell J, McDonald E, Lindroth R (2000) Effects of CO_2 and light on tree phytochemistry and insect performance *Oikos,* **88**, 259-272.

AICN (2008) Australian Insect Common Names. CSIRO and DAFF. http://www.ento.csiro.au/aicn/name

Ainsworth E, Long S (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist,* **165**, 351-372.

Alonso-Prados JL, Fraile A, Garcia-Arenal F (1997) Impact of cucumber mosaic virus and watermelon mosaic virus 2 infection on melon production in central Spain. *Journal of Plant Pathology,* **79**, 131-134.

Anderson P, Cunningham A, Patel N, Morales F, Epstein P, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution,* **19**, 535-544.

Anon (2000) Grower faces losses in millions. *The Bunyip.*23. Gawler, South Australia.

Awmack C, Harrington R, Leather S (1997) Host plant effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO_2. *Global Change Biology,* **3**, 545-549.

Awmack C, Harrington R, Leather S, Lawton J (1996) The impacts of elevated CO_2 on aphid-plant interactions In: Froud-Williams R, Harrington R, Hocking T, Smith H, Thomas T (eds). *Implications of Global Environmental Change for Crops in Europe - Aspects of Applied Biology (United Kingdom).* AAB, Cambridge UK pp.317-322.

Awmack CS, Harrington R, Lindroth RL (2004) Aphid individual performance may not predict population responses to elevated CO_2 or O_3. *Global Change Biology,* **10**, 1414-1423.

Bale J (2002) Herbivory in global climate research: direct effects of rising temperature on insect herbivores. *Global Change Biology,* **8**, 1-16.

Berg G (1984) The effect of temperature and host species on the population growth potential of the cowpea aphid, Aphis craccivora Koch(Homoptera: Aphididae). *Australian Journal of Zoology,* **32**, 345-352.

Bergant K, Trdan S, Žnidarčič D, Črepinšek Z, Kajfež-Bogata L (2005) Impact of climate change on developmental dynamics of *Thrips tabaci* (Thysanoptera: Thripidae): can it be quantified? *Environmental Entomology,* **34**, 755-766.

Bernklau E, Bjostad L (1998) Behavioural responses of first-instar western corn rootworm (Coleoptera: Chrysomelidae) to carbon dioxide in a glass bead bioassay. *Journal of Economic Entomology,* **91**, 444-456.

Best RJ (1968) Tomato spotted wilt virus. *Adv Virus Res,* **13**, 65-146.

Bezemer T, Jones T (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. *Oikos,* **82**, 212-217.

Bezemer T, Jones T, Knight K (1998) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid (*Myzus persicae*) and its parasitoid, *Aphidius matricariae*. *Oecologia,* **116**, 128-136.

Bezemer T, Knight K, Newington J, Jones T (1999) How general are aphid responses to elevated atmospheric CO_2? *Annals of the Entomological Society of America,* **92**, 724-730.

Blackman R, Eastop V (2000) *Aphids on the world's crops: an identification and information guide. 2nd Edn* John Wiley and Sons, Chicester, UK

BoM (2009) Average 9am and 3pm relative humidity maps. Australian Governement Bureau of Meterology http://www.bom.gov.au/climate/averages/climatology/relhum/IDCJCM0014_relative_humidity.shtml

Brittlebank CC (1919) Tomato diseases. *Journal of Agriculture, Victoria,* **17**, 231-235.

Butler GD (1985) Populations of several insects on cotton in open-top carbon dioxide enrichment chambers *The Southwestern Entomologist,* **10**, 264-267.

Butler GD, Kimball BA, Mauny JR (1986) Populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton grown in open-top field chambers enriched with CO2. . *Environmental Entomology,* **15**, 61-63.

Cammell ME, Knight JD (1992) Effects of climate change on the populations dynamics of crop pests. *Advances in Ecological Research,* **22**, 117-162.

Canto, T., Aranda, M.A. and Federes, A. (2009). Climate change effects on phydiology and population processes of hosts and vectors that influence the spread of hemipteran-borne plant viruses. Glogal change Biology 15:1884 - 1894.

Castle SJ, Perring TM, Farrar CA, Kishaba AN (1992) Field and laboratory transmission of watermelon mosaic virus 2 and zucchini yellow mosaic virus by various aphid species. *Phytopathology,* **82**, 235-240.

Cerkauskas R (2004) Tomato yellow leaf curl virus (TYLCV). *Fact Sheet.* Shanhua, Taiwan, AVRDC - The World Vegetable Center

Chapman RF (1988) *The Insects: Structure and Function (3rd edn),* Sevenoaks, London, Hodder and Stoughton.

Cho JJ, Mau RFL, Gonsalves D, Mitchell WC (1986) Reservoir weed hosts of tomato spotted wilt virus. *Plant disease. Nov,* **70**, 1014-1017.

Clift AD, Tesoriero L (2002) Aspects of vector thrips biology and epidemiology of tospoviruses in Australia. In: *Thrips and Tospoviruses: 7th International Symposium on Thysanoptera* Reggio, Calabria, Italy (eds Marullo R, Mound L) pp. 87-91. Canberra: Australian National Insect Collection CSIRO

Clover GRC, Smith HG, Azam-Ali SN, Jaggard KW (1999) The effects of drought on sugar beet growth in isolation and in combination with beet yellows virus infection. *Journal of Agricultural Science (Cambridge),* **133**, 251-261.

Cohen S1990) *Epidemiology of whitefly-transmitted viruses,* Newcastle upon Tyne, UK, Athenaeum Press 211-225pp.

Cohen S, Harpaz I (1964) Periodic, rather than continual acquistion of new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius) *Entomol. Exp. Appl.,* **7**, 155-166.

Cohen S, Lapidot M (2007) Appearance and expansion of TYLCV: a historical point of view. In: Czosnek H (ed) *Tomato yellow leaf curl virus disease. Management, molecular biology, breeding for resistance.* Springer.Dordrecht, Netherlands. pp.3-12.

Coll M, Hughes L (2008) Effects of elevated CO_2 on an insect omnivore: A test for nutritional effects mediated by host plants and prey. *Agriculture, Ecosystems and Environment,* **123**, 271-279.

Coutts B (2006) Virus diseases of cucurbit crops. Western Australia Department of Agriculture. Farmnote 166.

Coutts B, Jones R (2002a) Emergency vegetable growers meeting on TSWV and WFT in the Perth metro area. *National Management Strategy for the Management of Western Flower Thrips and Tomato Spotted Wilt Virus* **28**, 8.

Coutts B, Jones R (2005) Incidence and distribution of viruses infecting cucurbit crops in the Northern Territory and Western Australia. *Australian Journal of Agricultural Research,* **56**, 847-858.

Coutts BA, Thomas-Carroll ML, Jones RAC (2004) Patterns of spread of Tomato spotted wilt virus in field crops of lettuce and pepper: spatial dynamics and validation of control measures. *Annals of Applied Biology,* **145**, 231-245.

Coviella C, Trumble J (1999) Review: effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conservation Biology*, 700-712.

CSIRO, BoM (2007) *Commonwealth Scientific and Industrial Research Organisation and the Bureau of Meterology. Climate Change in Australia: Technical Report 2007*, CSIRO, Canberra, ACT. .

Czosnek H (2007) Interactions of tomato yellow leaf curl virus with its whitefly vector. In: Czosnek H (ed) *Tomato yellow leaf curl virus disease. Management, molecular biology, breeding for resistance.* Springer.Dordrecht, Netherlands. pp.157-170.

Czosnek H, Laterrot H (1997) A world-wide survey of tomato yellow leaf curl viruses. *Arch. Virol.,* **142**, 1391-1406.

Davidson RH, Lyon WF (1987) *Insect pests of Farm, Garden and Orchard,* New York, John Wiley and Sons.

De Barro PJ (1995) *Bemisia tabaci biotype B: a review of its biology, distribution and control. Division of Entomology Technical Paper. No. 36,* Canberra, ACT, CSIRO.

Desbiez C, Lecoq H (2004) The nucleotide sequence of Watermelon mosaic virus (WMV, Potyvirus) reveals interspecific recombination between two related potyviruses in the 5'part of the genome. *Archives of Virology,* **149**, 1619-1632.

Dioguardi D (1995) Lettuces. In: Coombes B (ed) *Horticulture Australia.* Morescope Publishing Melbourne.

Docherty M, Wade F, Hurst D, Whittaker J, Lea P (1997) Responses of tree sap-feeding herbivores to elevated CO_2. *Global Change Biology,* **3**, 51-59.

Donnelly A, Craigon J, Black CR, Colls JJ, Landon G (2001) Does elevated CO2 amerloirate the impact of O3 on chlorophyll content and photosynthesis in potato (*Solanum tuberosum*). *Physiologia Plantarium,* **111**, 510-511.

Drake B, Gonzàlez-Meler M, Long S (1997) More efficient plants: a consequence of rising atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology,* **48**, 609-639.

EPPO (2002) Diagnostic protocols for regulated pests *EPPO Bulletin* **32**, 281-292.

Faria M, Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. *Crop Protection,* **20**, 767-778.

Finnian JM, Donnelly A, Jones MB, Burke JI (2005) The effect of elevated levels of carbon dioxide on potato crops: a review *Journal of Crop Improvement,* **13**, 91-111.

Fleming R, Tatchell G (1995) Shifts in the flight periods of British aphids: a response to climate warming? In: Harrington R, Stork N (eds). *Insects in a Changing Environment.* Academic Press London. pp.505-508.

Fuhrer J (2003) Agroecosystem response to combinations of elevated CO_2, ozone, and global climate change. *Agriculture, Ecosystems and Environment,* **97**, 1-20.

Garrett RG, McLean GD1983) *The epidemiology of some aphid-borne viruses in Australia* Oxford, Blackwell Publishing 200-209pp.

Gerling D (1986) Natural enemies of *Bemisia tabaci*, biological characteristics and potential as biological control agents: a review *Agriculture, Ecosystems and Environment,* **17**, 99-110.

German TL, Ullman DE, Moyer JW (1992) Topsoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytopathology,* **30** 315-348.

Gibbs AJ, Mackenzie AM, Wei K-J, Gibbs MJ (2008) The potyviruses of Australia *Archives of Virology,* **153**, 1411-1420.

Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-preference in the butterfly *Coenonympha pamphuis* (Lepidoptera, Satyridae). *Global Change Biology,* **9**, 74-83.

Groves RL, Walgenbach JF, Moyer JW, Kennedy GG (2002) The role of weed hosts and tobacco thrips, Frankliniella fusca, in the epidemiology of Tomato spotted wilt virus. *Plant Disease,* **86**, 573-582.

Gunning RV, Bryne FJ, Condé BD, Connelly MI, Hergstrom K, Devonshire AL (1995) First report of B-Biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Journal of the Australian Entomolgical Society,* **34**, 116.

Hartz TK, Baameur A, Holt DB (1991) carbon diaoxide enrichment of high-value crops under tunnel culture *Journal of the American Society for Horticultural Science (USA),* **116**, 970-973.

Heagle AS (2003) Influence of elevated carbon dioxide on interactions between *Frankliniella occidentalis* and *Trifolium repens*. *Environmental Entomology,* **32**, 421-424.

Hill M (1994) *Western flower thrips,* Melbourne Institute of Horticultural Development, Knoxfield

Horlock C, Persley D (2004) Viruses affecting melons (watermelon, rockmelon and honeydew). Queensland Primary Industries and Fisheries. DPI&F Note

Horowitz AR, Podoler H, Gerling D (1984) Life table analysis of the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. *Acta Oecologica/ Oecologica Applicata,* **5**, 221-233.

Hughes L, Bazzaz FA (1997) Effect of elevated CO2 on interaction between the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the common milkweed, *Asclepias syriaca*. *Oecologia,* **109**, 286-290.

ICTVdBManagement (2006) *00.057.0.01.073. Watermelon mosaic virus. ,* New York, USA Columbia University.

Inoue T, Sakurai T, Murai T, Maeda T (2002) Accumulation and transmission of TSVW at larval and adults stages in six thrips species: distinct patterns between *Frankliniella* and *Thrips*. In: *Proceedings of the 7th International Symposium on Thysanoptera,* Universita' Degli Studi, Mediterranea di Reggio, Calabria (eds Marullo R, Mound L) pp. 59-65. ANIC Canberra.

IPCC (2007) Summary for Policymakers. In *Climate Change 2007: The Physical Science Basis.* Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel for Climate Change In: Solomon S, Qin D, Manning M, Xhen Z, Marquis M, Averyt K, Tignor M, Miller H (eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist,* **156**, 9-26.

Jelinek S (2009) NSW Vegetable IPM Newsletter - Integrated Pest Management for Insects and Viruses in Sydney Vegetables. Department of Primary Industries New South Wales

Johnson RH, Lincoln DE (1990) Sagebush and grasshopper responses to atmospheric carbon dioxide concentration. *Oecologia* **84**, 103-110.

Jones DR (2005) Plant viruses transmitted by thrips. *European Journal of Plant Pathology,* **113**, 119-157.

Jones R (2009) Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research,* **141**, 113-130.

Jui M, Zhou X-P, Tong L, Xu J, Yang X, Wan F-H, Liu S-S (2007) Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE,* **2**, 8pp.

Kimball B, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO_2 enrichment. In: Sparks D (ed) *Advances in Agronomy.* Academic Press Boston, US. pp.293-368.

Kläring HP, Hauschild C, Heißner A, Bar-Yosef B (2007) Model-based control of CO2 concentration in greenhouses at ambient levels increases cucumber yield. *Agricultural and Forest Meteorology,* **143**, 208-216.

Kopper B, Lindroth R (2003) Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO_2 and O_3. *Agricultural and Forest Entomology,* **5**, 17-26.

Layland J (1991) Thrips palmi. *AgNote.* Department of Primary Industry and Fisheries, Northern Territory.

Lenteren JCv, Noldus LPJJ1990) *Whitefly plant relationships: behavioural and ecological aspects.,* Andover, Harts, U.K., Intercept Ltd 47-89pp.

Lindroth RL, Roth S, Kruger EL, Volin JC, Koss PA (1997) CO_2-mediated changes in aspen chemistry: effects on gypsy moth performance and susceptibility to virus. *Global Change Biology,* **3**, 279-289.

Magliulo V, Bindi M, Rana G (2003) Water use of irrigated potato (Solanum tuberosum L.) grown under feree air carbon dioxide enrichment in central Italy. *Agriculture, Ecosystems and Environment,* **97**, 65-80.

Malipatil MB, Postle AC, Osmelak JA, Hill M, Moran J (1993) First record of *Frankliniella occidentalis* (Pergande) in Australia (Thysanoptera: Thripidae). *Journal Australian Entomological Society,* **32**, 378.

Maltby JE (1995) Tomatoes In: Coombes B (ed) *Horticulture Australia.* Morescope Publishing Melbourne.

Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience,* **37**, 110-118.

Miglietta F, Bindi M, Vaccari FP, Schapendock AHCM, Wolf J, Butterfield RE (2000) Crop ecosystem responses to climatic change: root and tuberous crops. In: Reddy KR, Hodges HF (eds). *Climate change and global crop productivity* CABI Publishing.New York. pp.189-209.

Moriones E, Navas-Castillo J (2000) Tomato yellow leaf curl virus, an emerging vcirus complex causing epidemics worldwide. *Virus Research,* **71**, 123-134.

Mound LA (1996) The Thysanoptera vector species of Tospoviruses. *Acta Horticulturae,* **431**, 298-309.

Nagata T, Almeida ACL, Resende RO, de Ávila AC (2004) The competence of four thrips species to transmit and replicate four tospoviruses (*Plant Pathology,* **53**, 136-140.

Nakićenović N, Swart R (2000) Special Report on Emissions Scenarios *A Special Report of Working Group III of the Intergovernmental Panel on Climate Change.* Cambridge, UK and New York, NY, USA, Cambridge University Press, Cambridge.

Namba R, Sylvester E (1981) Transmission of Cauliflower Mosaic Virus by the Green Peach, Turnip, Cabbage, and Pea Aphids. *Journal of Economic Entomology,* **74**, 546-551.

Naranjo S, Ellsworth P (2005) Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologica Experimentalis et Applicata,* **116**, 93-108.

Nelson MR, Tuttle DM (1969) The epidemiology of Cucumber mosaic and Watermelon mosaic 2 of Cantaloups in an arid climate. *Phytopathology,* **59**, 849-856.

Newman J, Gibson D, Hickam E, Lorenz M, Adams E, Bybee L, Thompson R (1999) Elevated carbon dioxide results in smaller populations of the bird cherry-oat aphid *Rhopalosiphum padi*. *Ecological Entomology,* **24**, 486-489.

Ng J, Falk B (2006) Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses.

Ng JCK, Perry KL (2004) Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology,* **5**, 505-511.

OEPP/EPPO (2005) *Tomato yellow leaf curl* and *Tomato mottle begomoviruses* PM7/50(1). *OEPP/EPPO Bulletin,* **35**, 319-325.

Oleson JE, Grevsen K (1993) Simulated effects of climate change on summer cauliflower production in Europe. *Europoan Journal of Agronomy,* **2**, 313-323.

Osborne CP, Mitchell PL, Sheehy JE, Woodward FI (2000) Modelling the recent historical impacts of atmospheric CO_2 and climate chane on Mediterranean vegetation. *Global Change Biology,* **6**, 445-458.

Osbourne JL, Awmack CS, Clark SJ, Williams IH, Mills VC (1997) Nectar and flower production in *Vicia faba* L. (field bean) at ambient and elevated carbon dioxide. *Apidologie,* **28**, 43-55.

Osbrink WLA, Trumble JT, Wagner RE (1987) Host suitability of *Phaseolus lunata* for *Trichoplusia ni* (Lepidoptera: Noctuidae) in controlled carbon dioxide atmospheres. *Environmental Entomology,* **16**, 639-644.

Pappu HR, Jones RAC, Jain RK (2009) Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus Research,* **141**, 219-236.

Parrella G, Cognalons P, Gebre-selassie K, Vovlas C, Marchoux G (2003) An update of the host range of *Tomato spotted wilt virus*. *Journal of Plant Pathology,* **85**, 227-264.

Pearson S, Wheeler TR, Hadley P, Wheldon AE (1997) A validated model to predict the effects of environment on the growth of lettuce (*Lactuca sativa* L.): implications for climate change. *Journal of Horticultural Science,* **72**, 503-517.

Peet M, Wolfe D (2000) Crop ecosystem responses to climate change: vegetable crops. In: Reddy K, Hodges H (eds). *Climate change and global crop productivity.* CABI Publishing Mississippi State University. pp.213-244.

Peñuelas J, Biel C, Estiarte M (1995a) Growth, biomass allocation, and phenology responses of pepper to elevated CO2 concentrations and different water and nitrogen supply. *Photosynthetica,* **31**, 91-99.

Persley DM, Thomas JE, Sharman M (2006) Tospoviruses - an Australian perspective. *Australasian Plant Pathology,* **35**, 161-180.

Picó B, Díez MJ, Nuez F (1996) Viral diseases causing the greatest economic losses to the tomato crop. II. The tomato yellow leaf curl virus - a review. *Scientia Horticulturae* **67**, 151-196.

Prange HD (1996) Evaoporative cooling in insects. *Journal of Insect Physiology,* **42**, 493-499.

ProMED, mail (2006) Yellow leaf curl, tomato - Australia (Qld): 1st Report. (Archive 20060330.0967) http://www.promedmail.org/pls/otn/f?p=2400:1001:::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C32517.03/07/09.

ProMED, mail (2009) Tomato yellow leaf curl virus - Australia (Queensland) (Archive No. 20090605.2079) http://www.promedmail.org/pls/otn/f?p=2400:1202:1358242140414859::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,77823.09/06/09.

Purcifull D, Hiebert E, Edwardson J (1984) Watermelon mosaic virus 2. *CMI/AAB Descriptions of plant viruses,* **293**.

QDPI&F (2005) Emerging plant pests - Melon thrips. www.dpi.qld.gov.au/health/2774.htm

QDPI&F (2006) Emerging plant pests - silverleaf whitefly. www.dpi.qld.gov.au/health/18449.htm

Reitz SR (2008) Comparative bionomic of *Frankliniella occidentalis* and *Frankliniella tritici*. *Florida Entomologist,* **91**, 474-476.

Reitz SR (2009) Biology and ecology of the Western Flower Thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomologist,* **92**, 7-13.

Reitz SR, Maiorino G, Olson S, Sprenkel R, Crescenzi A, Momol MT (2008) Integrating Plant Essential Oils and Kaolin for the Sustainable Management of Thrips

and Tomato Spotted Wilt on Tomato. *Plant disease: an international journal of applied plant pathology,* **92**, 878-886.

Riveroa RM, Sáncheza E, Ruiza JM, Romeroa L (2003) Influence of temperature on biomass, iron metabolism and some related bioindicators in tomato and watermelon plants *Journal of Plant Physiology,* **160**, 1065-1971.

Rubinstein G, Czosnek H (1997) Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: effect on the insect transmissioin capacity, longevity and fecundity. *Journal of General Virology,* **78**, 2683-2689.

Sakimura K (1962) The present status of thrips-borne viruses. In: Maramorosch K (ed) *Biological transmission of disease agents.* Academic, New York. pp.33-40.

Samuel G, Bald JG, Pittman HA (1930) Investigations on "spotted wilt" of tomatoes in Australia. *Australian council fo scientific and Industrial Research Bulletin,* **44**, 8-11.

Seal SE, vandenBosch F, Jeger MJ (2006) Factors influencing begomovirus evolution and their increasing global signficance: implications for sustainable control. *Critical Reviews in Plant Sciences,* **25**, 23-46.

Sherwood JL, German TL, Whitfield AE, Moyer JW, Ullman DE (2000b) *Tospoviruses,* New York, John Wiley and Sons.

Shukla DD, Ward CW, Brunt AA (1994) *The Potyviridae,* Wallingford, Oxon, CAB International

Siva-Subramaniam S, Lovatt J, Nolan B, Deuter P (2007) Silverleaf whitefly management in vegetable crops. Department of Primary Industries and Fisheries, Queensland http://www2.dpi.qld.gov.au/horticulture/18512.html.23/03/09.

Slack G, Fenlon JS, Hand DW (1988) The effects of summer CO2 enrichment and ventilation temperatures on the yield, quality and value of glasshouse tomatoes. *Journal of Horticultural Science,* **63**, 119-129.

Stange G (1992) High resolution measurement of atmospheric carbon dioxide concentration changes by the labial palp organ of the moth *Heliothis armigera* (Lepidoptera: Noctuidae). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology,* **171**, 317-324.

Stange G, Monro J, Stowe S, Osmond C (1995) The CO_2 sense of the moth *Cactoblastis cactorum* and its probable role in the biological control of the CAM plant *Opuntia stricta*. *Oecologia,* **102**, 341-352.

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. *Global Change Biology,* **13**, 1-20.

Stonor J, Hart P, Gunther M, DeBarro P, Rezaian MA (2003) Tomato leaf curl geminivirus in Australia: occurrence, detection, sequence diversity and host range. *Plant Pathology,* **52**, 379-388.

Taylor F (1981) Ecology and evolution of physiological time in insects. *American Naturalist,* **117**, 1-23.

Tesoriero L, Azzopardi S (2006) Tomato yellow leaf curl virus in Australia. Primefact 220, NSW Department of Primary Industries

Thomas J, Persley D (2007) Tomato yellow leaf curl virus. Department of Primary Industries and Fisheries, Queensland

Thompson G, Brown J, Woodward F (1993) The effects of host carbon dioxide, nitrogen and water supply on the infection of wheat by powdery mildew and aphids. *Plant Cell and Environment,* **16**, 687-694.

Van Brunschot, S.L., Persley, D. M., Geering, A.D.W., Campbell, P.R. and Thomas, J.E. (2010). *Tomato yellow leaf curl virus* in Australia: distribution, detection and discovery of naturally occurring defective DNA molecules. Australasian Plant Pathology 39: 412-423.

van de Wetering F, van der Hoek M, Goldbach R, Mollema C, Peters D (1999) Variation in tospovirus transmission between populations of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Bulletin of Entomological Research,* **89**, 579-588.

Varma A, Malathi VG (2003) Emerging geminivirus problems. A serious threat to crop protection. *Annals Applied Biology,* **142**, 145-164.

Veteli T, Kuokkanen K, Julkunen-Tiitto R, Roininen H, Tahvanainen J (2002) Effects of elevated CO_2 and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology,* **8**, 1240-1252.

Wagner TL (1993) Temperature-dependant development of sweetpotato whitefly *Bemisia tabaci* (Homoptera, Aleyrodidae) biotype "B". . *Proceedings of the Beltwide Cotton Conference,* **2**, 714-718.

Webb R, Scott H (1965) Isolation and identification of watermelon mosaic viruses 1 and 2. *Phytopathology,* **55**, 895-900.

Whittaker J (1999) Impacts and responses at population level of herbivorous insects to elevated CO_2. *European Journal of Entomology,* **96**, 149-156.

Wijkamp I, Almarza N, Goldbach R, Peters D (1995) Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology,* **85**, 1069-1074.

Wijkamp I, Lent Jv, Kormelink R, Goldbach R, Peters D (1993) Multiplication of tomato spotted wilt virus in its insect vector, *Frankliniella occidentalis*. *Journal of general virology. Mar,* **74**, 341-349.

Wijkamp I, Peters D (1993) Determinaton of the median latent period of two tospoviruses in *Frankliniella occidentalis,* using a novel leaf disk assay. *Phytopathology,* **83**, 456-463.

Williams R, Lincoln D, Norby R (1997) Effects of elevated CO_2, soil nutrient levels, and foliage age on the performance of two generations of *Neodiprion lecontei* (Hymenoptera: Diprionidae) feeding on loblolly pine *Environmental Entomology,* **126**, 1312-1322.

Wilson LJ, Bauer LR (1993) Species composition and seasonal abundance of thrips (Thysanoptera) on cotton in the Naomi Valley. *Journal of the Australian Entomolgical Society,* **32**, 187-192.

Wurr DCE, Edmondson RN, Fellows JR (2000) Climate change: a response surface study of the effects of CO_2 and temperature on the growth of French beans *Journal of Agricultural Science (Cambridge),* **135**, 379-387.

Wurr DCE, Hand DW, Edmondson RN, Fellows JR, Hannah MA, Cribb DM (1998) Climate change: a response surface study of the effects of CO2 and temperature on the growth of beetroot, carrots and onions *Journal of Agricultural Science (Cambridge),* **131**, 125-133.

Young G, Zhang L (1998) Control of the melon thrips, *Thrips palmi*. *AgNote.* Department of Primary Industry and Fisheries, Northern Territory.

Ziska LH, Bunce JA (2007) Predicting the impact of changing CO2 on crop yields: some thoughts on food. *New Phytologist,* **175**, 607-618.

Appendix

First record of Ranunculus white mottle virus from Australia

Gambley C.F., Persley D., Thomas J.E. and T Burfield

Since 2004, a disease of greenhouse capsicums has been observed in the North Adelaide Plains of South Australia. A vein-yellowing symptom was observed in affected plants and was similar to that described for Pepper yellow vein disease, a viral disease transmitted by the soilborne chytrid fungus *Olpidium* spp. (1). In 2008, stem sections of a fresh diseased sample (isolate 2155) was used to graft transmit the suspected virus to four cv. Yolo Wonder capsicum plants. The test capsicums developed vein-yellowing symptoms and in addition to isolate 2155 and a 2004 archived, lyophilised sample (isolate 1631) were tested by RT-PCR using the ophiovirus specific primers OP1 and OP2 specific for RNA-1 of the genus *Ophiovirus* (2). RT-PCR products of the expected 136 bp in size were obtained from all suspect virus-infected samples but not from healthy capsicum. To obtain a larger DNA fragment of the unknown virus for cloning and sequencing, a further degenerate genus-specific primer OP3 (TCDCAAACHCAAGTHACAAATGGAAG) was designed using the sequences of X, Y and Z (GenBank accessions X, Y and Z respectively). DNA fragments of *ca.* 1609 bp in size were amplified by RT-PCR using the primer OP2 with OP3 from isolate 1631 and one of the graft-inoculated capsicum plants. The DNA fragments were cloned and two clones from each amplicon sequenced. The consensus nucleotide sequence from the four clones was 97.4% and the -2 frame amino acid translation 98.7% identical to the Ranunculus white mottle virus (RWMV) GenBank accession QF335429. This represents the first report of RWMV infecting capsicum and of the presence of this virus in Australia.

When capsicum seedlings were planted into soil collected from under infected plants in the field and maintained in a growth cabinet set at 22° /20° C, 11 of 34 plants developed symptoms typical of yellow vein disease. Plants grown in pasteurised soil mix remained healthy.

1. Fletcher, J. T., Wallis, W. A., and Davenport, F. 1987. Pepper yellow vein, a new disease of sweet peppers. Plant Pathology 36:108-184.
2. Varia, A. M., Accotto, G. P., Costantini, A., and Milne, R. G. 2003. The partial sequence of RNA 1 of the ophiovirus *Ranunculus white mottle virus* indicates its relationship to rhabdoviruses and provides candidate primers for an ophiovirus-specific RT-PCR test. Archives of Virology 148 (1037-1050).

Appendix

Assessment of zucchini cultivars for tolerance to potyviruses

DM Persley and Lee McMichael

Inroduction

Zucchini cultivars were screened for tolerance to PRSV-W at Gatton in 2009. PRSV was selected as this virus has dominated virus surveys in south Queensland for at least five years and is a limiting factor to zucchini production in the Bundaberg, Lockyer valley and other areas of south Qld.

Methods

The trial was set up as four replicates, one row per replicate. There was a guard row on each side of the trial and a spreader row alternated with each data row. The spreader rows were squash cv. Green Ruffles and zucchini cv. Black Adder. There were 10 plants per plot .

The trial was hand sown with 4 to 5 seeds per location and then thinned soon after germination to 1 plant / plot. Plant spacing was 0.5 m.

Every second plant in the spreader rows was inoculated with PRSV-W by manual inoculation using sap / cold water and celite. Efficiency of this inoculation was 99%. Infection of data rows was via natural infection –virus was not introduced into the dataows by manual inoculation

Planted:	16 March 2009
Inoculated with PRSV:	27 March 2009
Harvest 1:	29 April 2009
Harvest 2:	6 May 2009
Harvest 3:	13 May 2009
Harvest 4:	27 May 2009

Collection of seed fruit and leaf samples for ELISA testing: 5 June 2009

Cultivar treatment and suppliers

Treatment	Company	Cultivar
1	SPS	Goldsmith
2	SPS	Litani
3	SPS	Nitro
4	SPS	Hummer
5	SPS	Dakota
6	Clause	Calida
7	Clause	Sintia
8	Clause	Amanda
9	Clause	Golden Arrow
10	SG	Crowbar
11	SG	Houdini
12	SG	Paydirt
13	Terranova	Mamba
14	Terranova	Black adder

Field Trial Layout

Replicate 1	Replicate 2	Replicate 3	Replicate 4
1-1 (7) Sintia	2-1 (1) Goldsmith	3-1 (8) Amanda	4-1 (2) Litani
1-2 (1) Goldsmith	2-2 (6)Calida	3-2 (6) Calida	4-2 (13) Mamba
1-3 (10) Crowbar	2-3 (7)Sintia	3-3 (12) Paydirt	4-3 (10) Crowbar
1-4 (6) Calida	2-4 (11)Houdini	3-4 (2) Litani	4-4 (4) Hummer
1-5 (9) Golden arrow	2-5 (13) Mamba	3-5 (13) Mamba	4-5 (1) Goldsmith
1-6 (1Black adder4)	2-6 (8) Amanda	3-6 (11) Houdini	4-6 (5) Dakota
1-7 (Paydirt12)	2-7 (4) Hummer	3-7 (5) Dakota	4-7 (12) Paydirt
1-8 (3)Nitro	2-8 (9) Golden arrow	3-8 (9) Golden arrow	4-8 (3) Nitro
1-9 (5)Dakota	2-9 (14) Black adder	3-9 (1) Goldsmith	4-9 (14) Black adder
1-10 (4)Hummer	2-10 (3) Nitro	3-10 (14) Black adder	4-1- (6) Calida
1-11 (13)Mamba	2-11 (5) Dakota	3-11 (7) Sintia	4-11 (8) Amanda
1-12 (11)Houdini	2-12 (10) Crowbar	3-12 (3) Nitro	4-12 (7) Sintia
1-13 (2)Litani	2-13 (2) Litani	3-13 (4) Hummer	4-13 (11) Houdini
1-14 (8)Amanda	2-14 (12) Paydirt	3-14 (10) Crowbar	4-14 (9) Golden arrow

Fruit of marketable size and greater were picked at each harvest, discarding fruit too large to market. All fruit with dead flowers even if a little small to be marketable were picked at harvest as well.

At each harvest leaf symptoms were rated, the number of marketable fruit and their weights recorded, along with the number of unmarketable fruit due to small size or deformity (due to virus infection or other causes eg. Insect damage) were counted and weighed. Large unmarketable fruit were counted separately and discarded.

Results

ELISA tests: Leaf samples collected randomly over field trial ensuring all varieties were sampled. All samples were positive for PRSV, 2 samples also positive for WMV, all samples negative for ZYMV.

Summary of symptom ratings and yields

Variety	Symptom rating (1 mild – 4 severe)	Total yield of marketable fruit	Per cent marketable fruit
Goldsmith	1	23.8 kg	62 %
Litani	3	6.2 kg	14 %
Nitro	3	20.0 kg	56 %
Hummer	4	2.0 kg	5 %
Dakota	4	2.5 kg	9 %
Calida	2	13.9 kg	40 %
Sintia	2	12.3 kg	39 %
Amanda	4	6.0 kg	17 %
Golden Arrow	1	25.7 kg	59 %
Crowbar	2	18.3 kg	58 %
Houdini	4	3.0 kg	9 %
Paydirt	2	16.9 kg	72 %
Mamba	4	4.4 kg	13 %
Black Adder	4	3.7 kg	16 %

Yield data calculated on total yields from 4 replicate trials of 10 plants each

Conclusions

There was a good correlation between virus severity ratings and yields of marketable fruit
The better performing cultivars were Paydirt, Goldsmith and Golden Arrow.