Australasian Plant Pathology (2023) 52:339–351
https://doi.org/10.1007/s13313-023-00925-9

ORIGINAL RESEARCH ARTICLE

Distribution and diversity of viruses affecting cucurbit production in New South Wales, Australia

S. Mulholland[1] · O. Wildman[2] · A. Daly[2] · L. Tesoriero[1] · T. A. Chapman[2]

Received: 10 May 2022 / Accepted: 28 May 2023 / Published online: 2 June 2023

Abstract

Cucurbits are an important crop grown across peri-urban, coastal and inland regions of New South Wales, Australia. Viral infection is a persistent issue across cucurbit commodities, different production methods and production regions. In this study, 34 cucurbit growing properties across five production regions of New South Wales were surveyed and sampled repeatedly from 2018 to 2021. Samples were tested for the presence of known endemic viruses using both serological and molecular diagnostic methods. Viral pathogens were detected on 22 of the 34 properties sampled, and in 44% of samples tested. Annual disease incidence ranged from 0 to 90%, typically increasing towards the end of the summer growing season. Papaya ringspot virus, watermelon mosaic virus, and cucumber mosaic virus, were identified as the most frequently detected viruses. Melon necrotic spot virus and beet pseudo yellows virus were detected at low rates. Cases of mixed infections of papaya ringspot virus and watermelon mosaic virus were also detected in some samples. Furthermore, cucumber green mottle mosaic virus, a "notifiable disease", was detected for the first time in New South Wales. A newly described virus, watermelon crinkle leaf associated virus-1, was also detected using next- generation sequencing technology. The latter two virus records represent a geographic range expansion and first report for Australia respectively.

Keywords Virus · Cucurbit · Biosecurity · Preparedness

Introduction

Viruses affecting cucurbit production are widespread throughout mainland Australian production regions and globally are a key limiting factor in cucurbit production (Lecoq and Desbiez 2012; Persley 2012). Viral infection negatively affects plant growth and can cause significant reductions in yield and product quality, leaving fruit deformed and unmarketable. Production is focused primarily on *Cucurbita pepo* (zucchini and squash), *C. moschata* and *C. maxima* (pumpkin), *Citrullus lanatus* (watermelon) and *Cucumis sativus* (cucumber). Typically, field cucurbit crops in New South Wales (NSW) are grown over the summer from September through to April whereas protected cropping (mainly cucumbers) may be grown all year round. The economic contribution of cucurbit commodities to Australia is valued at approximately $520 million annually (Horticulture Innovation Australia Limited 2020). Growers can experience severe economic impacts due to persistent disease outbreaks with incidences as high as 100% (Persley 2012).

For growers to apply effective management strategies they first need to understand the composition and incidence of viral populations across production areas. For instance, producers may select suitable plant varieties tolerant of key viral pathogens in their area. A challenging aspect of managing cucurbit viruses is their sheer number and diversity, in addition to a wide range of insect vectors and modes of transmission. Globally 194 distinct viruses have been characterized for the *Potyvirus* genus alone, over 35 of which have been detected in cucurbits to date around the world (Ward and Shukla 1991; Lecoq et al. 1998; Lecoq and Desbiez 2012; Sharma et al. 2016; ICTV 2022). At least 59 viruses from the dominant plant virus families have been detected in cucurbit production systems (Lecoq and Desbiez 2012).

✉ S. Mulholland
shannon.mulholland@dpi.nsw.gov.au

[1] NSW Department of Primary Industries, Plant Biosecurity Prevention and Preparedness, Ourimbah, NSW, Australia

[2] NSW Department of Primary Industries, Plant Biosecurity Research & Diagnostics, Menangle, NSW, Australia

In NSW, broad scale surveys of cucurbit pathogens are conducted infrequently. More commonly, samples with suspected viral infection are received by diagnostic laboratories on an ad hoc basis for pathogen determination, and a broader map of virus incidence is not generated through this process. This results in a disconnect between growers, diagnosticians and researchers. Prior to this study, the most recent regional survey in NSW was conducted in 2010-11 and focused on a relatively small grower group in the Sydney Basin (Persley 2012). At that time, surveys focused only on *C. pepo* (zucchini) production and tested for three prevalent endemic potyviruses (family *Potyviridae*): zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV) and papaya ringspot virus (PRSV). WMV and ZYMV were prevalent on the surveyed farms with incidence as high as 100%, whilst only low levels of PRSV were detected.

Based on survey results from other states, we expected that cucurbit viruses such as cucumber mosaic virus (CMV) (family *Bromoviridae*), would be largely absent from the NSW production areas due to a lack of detection in other production regions (Persley 2012; Coutts and Jones 2005). CMV was not specifically screened for during the last NSW survey so true incidence levels were unknown. Cucumber green mottle mosaic virus (CGMMV) is a notifiable disease in NSW but the true incidence of this virus across the state was unclear. CGMMV is a highly transmissible cucurbit virus responsible for significant crop losses particularly for field grown cucurbits. CGMMV belongs to the family *Virgaviridae*, genus *Tobamovirus*, and can induce yield reduction, fruit discolouration, and deformation. The internal quality of the fruit can be so severely impaired as to render the fruit unmarketable (Dombrovsky et al. 2017). Verifying if CGMMV was present in NSW was a critical aspect to this study as surveillance data underpins support for market access. Several NSW growers export their product internationally and it is a requirement of certain trade agreements that the property demonstrate freedom from CGMMV infection. Broader regional surveys for this pathogen may assist this process by demonstrating area freedom.

Cucurbits such as zucchini are predominantly summer crops in NSW, and producers need to understand where viruses are overwintering in the landscape (Jones 2004; Desbiez and Lecoq 1997). Vegetable producers, particularly around the Sydney Basin, persist on the peri-urban fringe. A diverse mosaic of land uses surrounds these properties presenting a complex vegetation community of garden ornamentals, weed species, native vegetation, and other vegetable crops. This situation provides an abundant resource of host plants to harbor both cucurbit viral pathogens and their insect vectors (Lecoq and Desbiez 2012; Dombrovsky et al. 2017; Desbiez and Lecoq 1997). These virus reservoirs are poorly understood by growers and not typically incorporated into current disease management programs. Research efforts to identify new host species have largely been conducted overseas and there is a real need to verify the host range in an Australian context, particularly concerning Australian native or naturalised species (Tessitori et al. 2002; Lecoq and Desbiez 2012; Gibbs et al. 2008). Another key objective of this project was to identify common weed species hosting cucurbit viruses. Knowledge of where viruses can be found in high densities within a landscape can assist producers in managing common viral host species (beyond their cropping varieties).

Our study aimed to conduct surveillance for a broad range of cucurbit viruses across numerous production areas of NSW. Key surveillance targets included zucchini, squash, cucumber, pumpkin and melon host crops to provide a contemporary snapshot of viral populations. The objective was to verify the etiology of viruses impacting cucurbit production, understand virus incidence across geographic regions and confirm the absence of CGMMV in field grown melon crops. An assessment of alternative plant hosts was incorporated to better understand how the NSW viral population persists within the landscape in the absence of summer cucurbit crop hosts. As part of a contribution to a larger national screening process for endemic viruses several symptomatic cucurbit samples collected in NSW during this survey were also subjected to next generation sequencing (NGS). These samples had returned a positive result for one of the target viruses of this survey and the intention of the NGS was to further contribute to the knowledge base of the diversity of Australian viral pathogens.

Materials & methods

Surveys of both field grown and protected cropping cucurbit production areas across NSW were undertaken between September 2018 and November 2021. The primary target was zucchini, however, where possible, samples were taken from pumpkin, squash, cucumber, rockmelon and watermelon crops. Host plants such as weeds and other vegetable crops were sampled from within, or in close proximity to, symptomatic cucurbit crops to identify alternative virus hosts (Table 1 lists all species sampled). Alternative plant hosts were typically present at low densities therefore, at least 3 specimens per species were collected where possible, but incidence rates (of observable virus symptoms) for this cohort were not recorded.

Surveys were conducted using a standardized protocol where a minimum of 300 plants per crop (6 randomly selected blocks of 50) were visually inspected and the percentage with observable viral disease symptoms recorded. Crops were walked in a W- or Z-pattern to include edges

Table 1 Full list of host species sampled during surveillance activities

Weed species	Vegetable and other species
Amaranth spp.	*Abelmoschus esculentus* (okra)
Anagallis arvensis (scarlet pimpernel)	*Beta vulgaris* (beetroot, silverbeet)
Araujia sericifera (moth vine)	*Brassica rapa* (mizuna, wombok)
Asparagus asparagoides (bridal creeper)	*Capsicum annuum* (chilli)
Brassica rapa (turnip weed)	*Citrullus lanatus* (watermelon)
Cardiospermum grandiflorum (balloon vine)	*Cucumis sativus* (cucumber)
Capsella bursa-pastoris (shepherds purse)	*Cucumis melo* (rockmelon)
Cestrum parqui (green cestrum)	*Cucurbita pepo* (zucchini, squash, spaghetti squash)
Chenopodium album (fat hen)	*C. moschata/ C. maxima* (pumpkin, potkin)
Conyza bonariensis (fleabane)	*Cynara cardunculus (*artichoke)
Cucumis myriocarpus (paddymelon)	*Lactuca sativa* (lettuce, Chinese cabbage)
Datura stramonium (thornapple)	*Limonium carolinianum* (sea lavender)
Echium plantagineum (paterson's curse)	*Lobelia spp.*
Foeniculum vulgare (fennel)	*Lupinus spp.* (lupins)
Jacobaea vulgaris (ragwort)	*Medicago sativa* (lucerne)
Lactuca serriola (prickly lettuce)	*Phaseolus vulgaris* (bean)
Lantana camara (lantana)	*Raphanus sativus* (daikon radish)
Lycium ferocissimum (African boxthorn)	*Rheum spp.* (rhubarb)
Malva spp. (mallow weed)	*Solanum melongena* (eggplant)
Onopordum acanthium (Scotch thistle)	*Solanum tuberosum* (potato)
Physalis acutifolia	*Typha orientalis* (typha)
Phytolacca octandra (inkweed)	*Vicia faba* (broadbean)
Portulacca oleracea (purslane)	
Raphanus raphanistrum (wild radish)	
Rumex spp. (dock)	
Senecio madagascarensis (fireweed)	
Sida rhombifolia (Paddy's lucerne)	
Solanum nigrum (blackberry nightshade)	
Sonchus oleraceus (sowthistle)	
Stellaria media (chickweed)	
Tribulus terrestris (caltrop)	
Verbena bonariensis (purpletop)	
Xanthium spinosum (Bathurst burr)	

and internal sections of the block. Symptomatic plants were sampled for further analysis to confirm the pathogen identity, with at least three leaf samples of each symptom collected and a minimum of three asymptomatic leaves from each crop. Both symptomatic and asymptomatic alternative hosts were sampled. Farms were typically less than 20 ha therefore, all available cucurbit crops per farm were surveyed on each visit. Additionally, aphid vectors were periodically collected in ethanol and identified to species during the survey period. Aphids were collected from within symptomatic cucurbit crops and immediately transferred to 70% ethanol for preservation. Morphological identification of the aphids was performed by the Biosecurity Collections Unit at the NSW Department of Primary Industries Orange Agricultural Institute.

There were five primary viral pathogens targeted for screening during the surveillance period: ZYMV, WMV, PRSV, CMV and CGMMV. In addition, several symptomatic cucurbit samples that did not return a positive result for the virus panel just described were subjected to additional testing to determine the causal agent. This included specific investigation for melon necrotic spot virus (MNSV) beet pseudo yellows virus (BPYV) based on observed symptoms, and confirmatory diagnostics for watermelon crinkle leaf associated virus-1 (WCLaV-1) in response to its detection during Next Generation Sequencing (see below).

Serological diagnostics

Serological screening was performed for target pathogens CMV, PRSV, WMV, and ZYMV. Leaf samples (approximately $2cm^2$) were placed in 2 mL tubes with a stainless-steel ball bearing and 400 µL of buffer before being homogenized using a Qiagen TissueLyser™ (QIAGEN, Hilden, Germany) at 50 Hz/s for 2 min. Samples were screened by the standard double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) [Cat. No.: WMV – SRA 54,001/0096] and Agdia Pathoscreen™ kits, in accordance

with the manufacturer's instructions (Agdia Inc., Elkhart Indiana, USA) [Cat. No.: CMV – PSA 44,501/0096; PRSV – PSA 53,500/0096; ZYMV – PSA 77,700/0096]. Samples with absorbance values (read at 405 nm) greater than twice the mean of negative controls were considered positive (EPPO 2015). Cucurbit samples from each farm surveyed were pooled (maximum of 5 leaves) and tested for CGMMV using the lateral flow Agdia ImmunoStrip® test for CGMMV, as part of the sample triaging process, as per the manufacturer's instructions (Agdia Inc.). Samples with positive ImmunoStrip® results or symptoms consistent with CGMMV were subjected to further serological and molecular analyses. Serological diagnostics were performed using two CGMMV specific DAS-ELISA kits (either singly or in tandem) as per the manufacturer instructions (Agdia Inc.; Prime Diagnostics, Wageningen University & Research, Netherlands). For MNSV, serological diagnostics were performed using a specific DAS-ELISA assay as per the manufacturer's instructions (Agdia Inc.).

RT-PCR diagnostics

For molecular confirmation, samples were progressed through to end-point or real time reverse transcription polymerase chain reaction (RT-PCR) using the primer sets and protocols described in Table 2. Total RNA was extracted from symptomatic leaf tissue using commercially available column-based extraction methods (RNeasy Plant Mini Kit (Qiagen, Germantown, Maryland, USA) and the Invitrogen/Ambion PureLink RNA Mini Kit (Invitrogen, Waltham, Massachusetts, USA)), according to the manufacturer's instructions. Invitrogen SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase was used for the end-point RT-PCR assays (Invitrogen, Waltham, Massachusetts, USA). For real-time RT-PCR CGMMV analysis, the QuantaBio qScript XLT 1-Step RT-qPCR ToughMix™ (QuantaBio, Berverly, Massachusetts, USA) or RNA UltraSense™ One-Step Quantitative RT-PCR System (Life Technologies, Frederick, Maryland, USA) kits were used.

The purified RT-PCR products were sequenced in both directions at the Australian Genome Research Facility Ltd (Sydney, Australia), with automated sequencing using an Applied Biosystems 3730xl capillary sequencer (www.agrf.org.au). Sequences were quality checked and aligned using Geneious Prime 2021 (www.geneious.com).

NGS library preparation

As an additional screening mechanism next generation sequencing (NGS) using the Illumina™ platform was applied to six randomly selected watermelon samples with the assistance of Agriculture Victoria Research at AgriBio in Victoria, Australia, followed by additional RT-PCR verification using the protocol outlined in Xin et al. 2017. Total RNA was extracted from leaves of symptomatic watermelon plants using the RNeasy Plant Mini Kit (Qiagen). Samples were subjected to library preparation using the NEBNext® Ultra™ RNA Library Prep Kit (New England BioLabs, Ipswich, Massachusetts, USA) following the manufacturer's instructions. The library was sequenced using MiSeq cycle

Table 2 RT-PCR protocols used to detect viruses in plant samples

Pathogen	Target	Primer names	Protocol reference
Potyvirus (PRSV, WMV, ZYMV)	Nib (replicase gene)	U341-F, Poty1-R	Harper et al. 2019 using the primers described in Gibbs and Mackenzie 1997 and Langeveld et al. 1991, performed as a One-Step RT-PCR
Cucumovirus (CMV)	Coat protein	CPTALL-5, CPTALL-3	Choi et al. 1999
CGMMV	Movement protein Coat protein	MP F1, UTR R1 MP-F, MP-R CP-F, CP-R	Ling et al. 2014
	Coat protein Movement protein	CP-F, CP-R MP-F, MP-R	Reingold et al. 2015
	CGMMV genome position 62-1115	F-62, R-1115	Shargil et al. 2019
	3' Noncoding region (NCR)	CGMMV-sense, CGMMV-antisense, CGMMV-probe	Hongyun et al. 2008
	Movement protein	RZ_CGMMVmp01, RZ_CGMMVmp03, RZ_CGMMVmp04	Berendsen and Oosterhof 2015
WCLaV-1	Movement protein	MP-1 F, MP-1R MP-2 F, MP-2R	Xin et al. 2017
	Nucleocapsid protein	NP-1 F, NP-1R NP-2 F, NP-2R	
BPYV	Minor coat protein	BP CPm F, BP CPm R	Tzanetakis and Martin 2004

2×251 bp (Illumina, San Diego, California, USA) to generate paired-end reads. The NGS raw sequence reads were trimmed using Trim galore (Version 0.6.4) (Krueger 2012), followed by *de novo* assembly using SPAdes (Version 3.14.0) (Bankevich et al. 2012). For homology confirmation the *de novo* assembly contigs were analysed using BLASTn (Altschul et al. 1997).

Results

Thirty four individual farms were included in the surveys. During the survey period, 85 farm visits across 5 regions were conducted with a total of 284 crop inspections (most properties were surveyed more than once and multiple crops were inspected on each farm, each recorded as a separate crop inspection). Most properties surveyed were in the Sydney Basin (24), but properties were also surveyed in the Central Coast (2), Riverina (1), Hunter (2), and Sunraysia (5) regions of NSW (Fig. 1). Cucurbit viruses were detected in all 5 NSW production regions surveyed and the virus population dynamic was noted to vary by region (Tables 3 and 4).

Cucurbit viruses and hosts identified during the surveillance are listed in Table 4. A total of 224 cucurbit, and 390 weed and other vegetable crop samples were collected from 284 crop inspections between 2018 and 2021. Viral pathogens were detected in 44% of cucurbit samples using serological and molecular screening methods. From the targeted screening panel used in this survey CMV, PRSV and WMV were detected in 19% of all plant samples tested (i.e. cucurbit, vegetable and weed hosts) and were identified on 22 of 34 properties surveyed.

When only considering the cucurbit samples, WMV had the highest overall prevalence (46% of virus positive samples) followed by PRSV (21%) and CMV (8%). WMV and CMV had the highest frequency of detection across all farms and regions surveyed, recorded on 11 of 34 properties respectively, in both cucurbit and alternative plant hosts. The highest concentration of infection was recorded within the

Fig. 1 Location of surveyed NSW cucurbit production regions

Table 3 Viruses detected in cucurbit crops in vegetable production zones of NSW during 2018–2021 surveys

Region	No. farms with virus detected/ total no. farms surveyed	No. site visits/no. crop inspections	Range of virus incidence (%)	Viruses detected (No. farms with each virus)	Cucurbit crops surveyed
Sydney Basin	15/24	71/238	WMV- 0–90% PRSV- 0–90% CMV- 0–75%	WMV (9) PRSV (5) PRSV + WMV (3) CMV (10) CGMMV (1) BPYV (1)	Zucchini, squash, cucumber, rockmelon, watermelon, potkin, pumpkin
Central Coast	2/2	6/14	WMV- 0-0.25% CGMMV- 0–70%	WMV (1), CGMMV (1)	Cucumber, Zucchini
Hunter	1/2	2/12	CMV – 100%*	CMV (1)	Cucumber
Sunraysia	2/5	5/12	WMV- 25% WCLaV-1- 5-25% CGMMV- 0–25%	WMV (1) WCLaV-1 (1) CGMMV (2)	Watermelon, zucchini, pumpkin
Riverina	1/1	1/8	MNSV – 0–5%	MNSV (1)	Watermelon

*represented a single plant

Table 4 Cucurbit virus detections by host during 2018–2021 NSW surveys

Plant Host	No. Samples collected	No. of positive detections						
		WMV	PRSV	CMV	CGMMV	WCLaV	BPYV	MNSV
Cucumber *(Cucumis sativus)*	19	-	-	1	3	-	**1**	-
Potkin (*Cucurbita maxima)*	1	1	-	-	-	-	-	-
Pumpkin (*Cucurbita maxima)*	11	3	-	1	-	-	-	-
Rockmelon (*Cucumis melo)*	2	1	-	-	-	-	-	-
Spaghetti squash (*Cucurbita pepo)*	1	-	-	1	-	-	-	-
Squash (*Cucurbita pepo)*	8	2	2	-	-	-	-	-
Watermelon (*Citrullus lanatus)*	55	3	-	-	2	4	-	**7**
Zucchini (*Cucurbita pepo)*	144	45	22	7	-	-	-	-
Broadbean (*Vicia faba)*	8	-	-	1	-	-	-	-
Bean (*Phaseolus vulgaris)*	4	-	-	2	-	-	-	-
Blackberry nightshade (*Solanum nigrum)**	6	-	-	1	-	-	-	-
Fleabane (*Conyza bonariensis)**	6	-	4	1	-	-	-	-
Moth vine (*Araujia sericifera)**	13	-	-	4	-	-	-	-
Purslane (*Portulaca oleracea)**	4	-	-	1	-	-	-	-
Sowthistle (*Sonchus olearaceus)**	8	-	1	1	-	-	-	-
Thornapple (*Datura stramonium)**	6	1	-	-	-	-	-	-
Turnip weed (*Brassica rapa)**	15	-	-	1	-	-	-	-
Total Number of Positive Detections		**56**	**29**	**22**	**5**	**4**	**1**	**7**

* Non-crop host

Sydney Basin, a direct correlation with the large number of vegetable growers surveyed in this region. Mixed infections of PRSV and WMV were detected in 8 zucchini samples from 2 separate properties. ZYMV was not detected in any plant sample during the entire survey period. MNSV was detected in 7 watermelon samples from a single property and BPYV was detected in 1 cucumber sample from a single property. CGMMV was detected on two properties growing greenhouse cucumbers during the survey period, one on the Central Coast and one in the Sydney Basin, and on a further two melon growing properties in the Sunraysia region. These are the first reported detections of CGMMV in NSW. Samples from all four affected properties produced a positive reaction with the Agdia CGMMV ImmunoStrip® tests as did the DAS-ELISA. RT-PCR analysis detected amplicons of the expected size for CGMMV. BLASTn analysis to the NCBI database revealed that different isolates exhibited homology to various accessions (Table 5), both within Australia and internationally.

Records of disease incidence in the field were based on observable symptoms and ranged from 0 to 90%. Virus detections peaked in May 2019, and incidence typically increased towards the end of the growing season. The individual breakdown of incidence per virus for each production area is shown in Table 3.

Several different zucchini cultivars were assessed during the surveillance period. Observable incidence of virus symptoms varied between cultivars and between seasons.

Table 5 CGMMV detections in NSW

Isolate	Host	Location	Highest % blast similarity	Origin of GenBank isolate
AWM0504	Cucumber	Central Coast	99.78% to MH427279.1 CGMMV GenBank accessions	Northern Territory, Australia
AWM0775	Watermelon	Sunraysia	99.74% identity to KY115174.1 CGMMV GenBank accession	Western Australia, Australia
AWM0776	Watermelon	Sunraysia	99.74% to MH271442.1 GenBank accession	USA
AWM0777	Cucumber	Sydney Basin	99.74% to MH271419.1 GenBank accessions	Netherlands

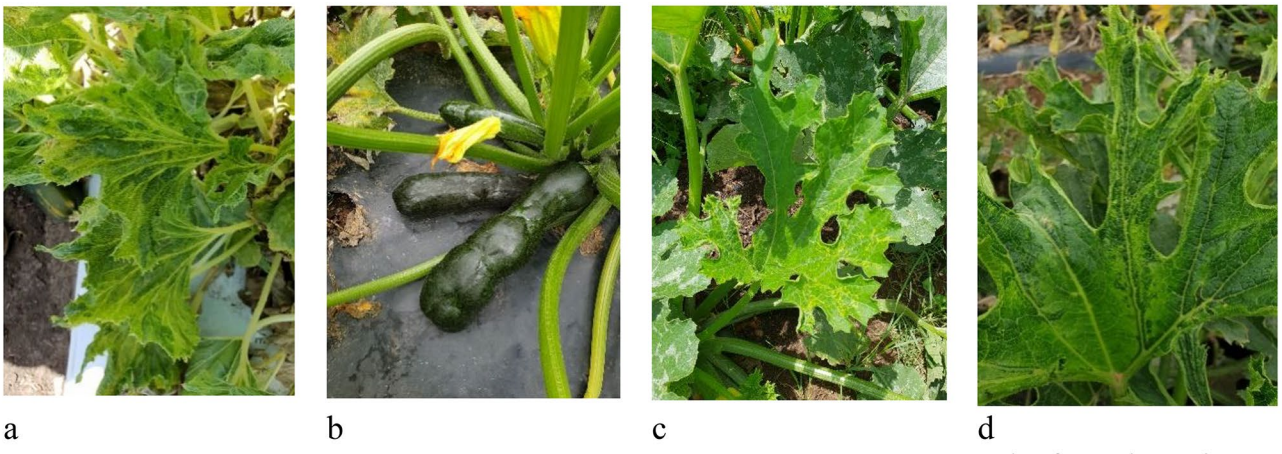

Fig. 2 Typical viral symptoms encountered in NSW surveys: **a** - PRSV leaf mottle and deformation in squash; **b** - PRSV fruit deformation in zucchini; **c** – CMV leaf mosaic in zucchini; **d** – WMV leaf mottle and deformation in zucchini

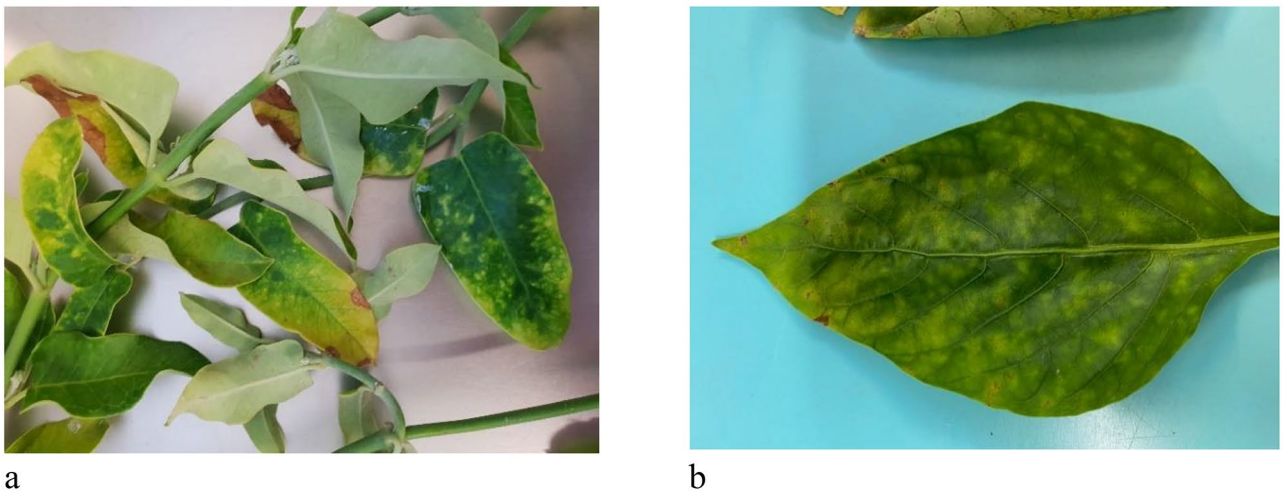

Fig. 3 Viral symptoms of CMV detected in alternative plant hosts in NSW surveys: **a** - CMV leaf mottle and yellow discoloration in moth vine; **b** - CMV leaf mottle in bean

The two dominant cultivars of zucchini 'Rosa' and 'Eva' recorded disease incidence of 0–82% and 0–90% respectively. A newer zucchini cultivar, cv. 'Nitro' had very low virus incidence (0–2%) based on observable symptoms, however it should be noted that the surveyed blocks were planted early in the growing season, ahead of virus peaks. Another new cultivar being trialed by some growers, cv. 'Apollonia' exhibited a similarly low level of viral symptom expression to that of 'Nitro'.

Typical symptoms of the common viruses encountered during the NSW surveys can be found in Figs. 2 and 3. WMV and CMV affected zucchini and pumpkin plants exhibited leaf chlorosis, faint mosaic and leaf distortion, and were considered mild to moderate in severity. Some fruit discoloration and deformity was observed, particularly for WMV affected plants. Alternatively, PRSV induced severe symptoms such as leaf bubbling/rugosity, discolouration, necrosis, severe leaf distortion and fruit symptoms including

lumps, distorted growth and discoloration. One grower experienced successive PRSV outbreaks towards the end of the 2018–2019 growing season and reported around 30% yield loss from this virus alone in his zucchini crops. Some WMV and CMV viral infections were noted to be asymptomatic. CGMMV symptoms on cucumber included severe leaf mottle, leaf bubbling/rugosity and downward curling (Fig. 4). Some fruit exhibited faint blotches across the skin surface. The watermelon samples exhibited necrotic lesions on the stem and peduncle of the fruit.

A total of 55 alternative host species were sampled during this project with virus positive results presented in Table 4. For alternative plant hosts (i.e. all other weed and non-cucurbit crop species), 17% of the samples tested positive for at least one virus. CMV was the most frequently encountered virus in non-cucurbit alternative hosts (12% of virus positive samples). The frequency of potyviruses was noticeably lower in non-cucurbit hosts, with WMV and PRSV detected in 2% and 5% of virus positive samples respectively. For *Araujia sericifera* (moth vine), 30% of collected samples tested positive for CMV. This weed species was observed as highly prevalent across the Sydney Basin, particularly in disturbed areas. Symptoms on weeds ranged from asymptomatic to leaf mottle, bubbling on leaves and stunted growth. The following species were the most common weed species observed across NSW production areas: *Araujia sericifera* (moth vine), *Datura stramonium* (thornapple), *Sonchus olearaceus* (sowthistle), *Malva spp.*(mallow), *Portulaca oleracea* (purslane), *Conyza bonariensis* (fleabane), *Solanum nigrum* (blackberry nightshade), *Bidens pilosa* (farmers friends), and *Brassica rapa* (turnip weed).

Symptomatic watermelon leaf tissue was collected from the Sunraysia region during surveillance activities in 2019. Following RT-PCR, an amplicon of the correct size for a potyvirus was detected from the original AWM0161 sample. BLASTn analysis identified a 96% homology to various WMV accessions lodged in GenBank. This isolate was then subjected to NGS for further analysis. It was at this point that full genomes of watermelon crinkle leaf associated virus-1 (WCLaV-1) and WMV were identified (deposited in GenBank under accessions OM884315 and OM884316). Confirmatory RT-PCR returned a positive result for WCLaV-1 on this isolate. This property was revisited and additional samples of watermelon collected. Further RT-PCR again detected single infections of WCLaV-1. The WCLaV-1 isolate had a high sequence homology to watermelon isolates from China (Mulholland et al. 2022).

Aphid vectors

Three species of aphids were identified during the surveillance and are listed in Table 6 along with the viruses they are known to vector and those that were detected during the field surveys.

Discussion

Farms included in the surveillance were typically 10–20 ha and grew an array of vegetable crops year-round. Melon growers in the Riverina and Sunraysia regions typically grew a mix of rockmelons and watermelons with field crops

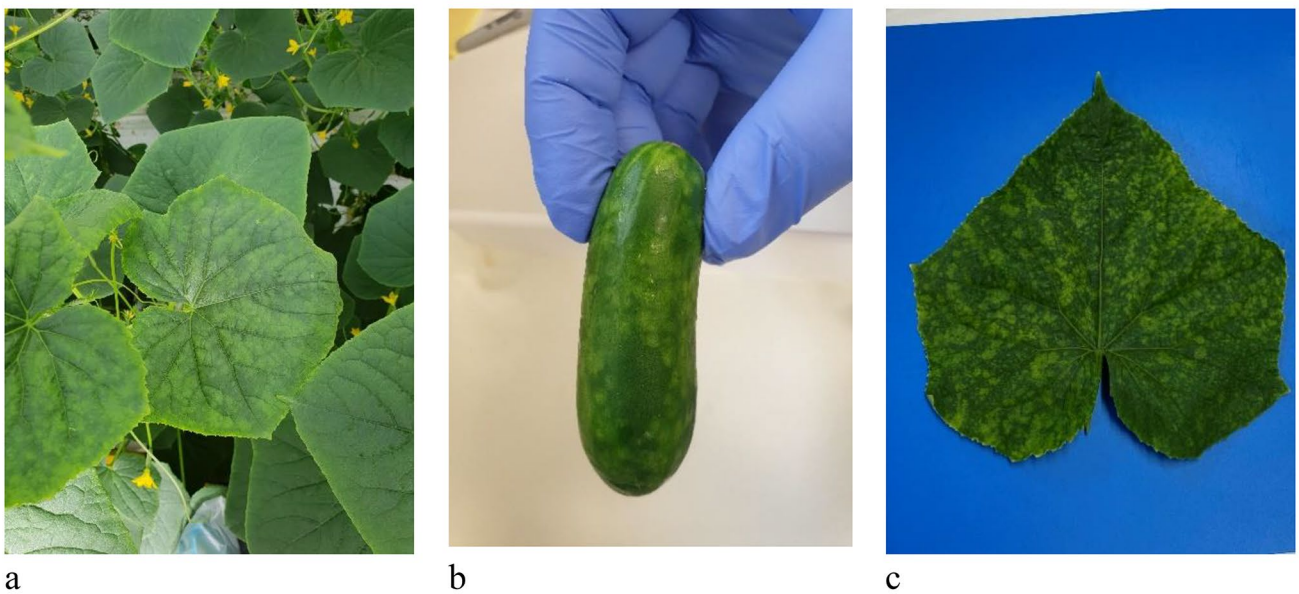

Fig. 4 CGMMV symptoms observed in cucumber during NSW surveys: **a** - leaf mottle and downward curling of leaves; **b** - mottle and dimpling in cucumber fruit; **c** - leaf mosaic

Table 6 Aphid species detected during NSW field surveys

Species	Common name	Known virus vector	Host plant
Aphis gossypi (Glover)	Melon/cotton aphid	Yes – potyviruses, CMV	Sowthistle (*Sonchus oleraceus*), zucchini
Aulacorthum solani (Kaltenbach)	Foxglove/glasshouse-potato aphid	Yes – potyviruses, CMV	Zucchini, pumpkin
Myzus persicae (Sulzer)	Green peach aphid	Yes – potyviruses, CMV	Zucchini, pumpkin

often exceeding 20–40 ha. Many of the surveyed peri-urban properties were bounded by small rural landholdings and encroaching suburbia, selling to local farmers' markets and central markets in Sydney, Melbourne, Brisbane and Newcastle. Larger melon farms have markets in most Australian states and a small volume is exported to New Zealand, Singapore, United Arab Emirates and Japan (Horticulture Innovation, 2020).

The findings from this surveillance program indicate that the population of viral pathogens causing economic losses to cucurbit growers, particularly around the Sydney Basin, has altered over time when compared to the findings of the last survey of NSW cucurbit commodities some 11 years ago (Persley 2012). Virus incidence and diversity was highest in the Sydney Basin compared with other regions surveyed although this is likely an outcome of the large number of growers and subsequent crop inspections undertaken in this region. WMV was identified as the dominant viral pathogen detected across NSW cucurbit production areas during our survey and currently PRSV is far more abundant when compared with the 2011 survey data. CMV is more prevalent within NSW cucurbit production areas than first thought. This may be in part due to the lack of targeted screening for this virus during previous surveys, or seasonal differences in weather and associated virus phenology in alternative hosts. Historical records sourced from the Australian Plant Pest Database (APPD, 2019) illustrate that CMV has been recorded across a broad range of hosts in NSW since 1961, with the primary cucurbit host record from cucumber.

ZYMV was not detected in any of the samples during this survey period. This was a surprising outcome as it was one of the most prevalent viruses detected during previous large-scale NSW surveys (Persley 2012; Pilkington 2011). The APPD database holds a single record of ZYMV from the Sydney Basin in a cucumber host from 2001. It is unclear whether the absence of ZYMV during this survey period is due to natural variation in the population or that its environmental niche has been encroached upon by other viruses such as PRSV or CMV. Also, it is plausible that ZYMV tolerant varieties have gradually reduced the inoculum load of this virus in the landscape to a point below significant environmental and economic damage to production.

Symptom expression did vary on virus-infected zucchini. Cultivars 'Eva' and 'Rosa' currently constitute the major zucchini cultivars grown in the Sydney Basin. Historically, both cultivars have claimed intermediate tolerance to potyviruses (Clause 2015). Given the high rates of potyvirus detection and severe symptoms observed on infected plants, particularly the impact to marketable fruit, it may indicate that the tolerance is now less effective against the current potyvirus variants circulating in commercial crops and growers may need to assess the need for including additional cultivars into their crop management rotation.

Virus incidence typically did not peak until late in the summer growing season. Additionally, asymptomatic infections were encountered, particularly for WMV, indicating that visual surveys may be underestimating the true incidence of virus infection. Cucurbit viruses are commonly detected at low levels soon after the first crops are planted in spring. However in 2018, cucurbit viruses were not detected until the end of December and viruses were not frequently intercepted that season until February. Aphid numbers were also observed to be abnormally low for the early part of the 2018 growing season, possibly owing to the prolonged severe drought experienced by much of NSW at that time (BOM 2022). Rainfall around February 2019 correlated with an increase in virus and aphid vector detections. The mild winter of 2019 saw cucurbit production extend far into May and resume again in early August. Furthermore, mild winter conditions contributed to elevated numbers of aphid vectors early in the 2019-20 growing season and virus detections were correspondingly also much earlier than the previous year (October 2019 compared to December 2018). Three common aphid species were detected during the surveillance. All three species are known viral vectors although their transmission efficacy differs (Contangelo et al. 1994; Marino et al. 2010).

The detection of CGMMV in key production zones is a concerning development for the vegetable and melon industries of NSW. This is the first detection of CGMMV in NSW, and a geographic range expansion in Australia. This infection was found in different hosts across a wide geographic range which may indicate there have been multiple introductions of this virus into NSW. Investigations are ongoing to understand the transmission pathway and source of infection, be it from within Australia or an international source via seed. This virus was first detected in Australia in the Northern Territory in 2014 and has since been detected in Western Australia, South Australia and Queensland (Dombrovsky et al. 2017; Tesoriero et al. 2016). CGMMV is a particularly stable virus persisting on hard surfaces for long periods (Reingold et al. 2015). Its high transmission

efficacy can rapidly spread infection throughout a crop and management is problematic as it can persist in dead vegetative material, water and soil for long periods of time (Choi et al. 2004; Li et al. 2016; Park et al. 2010). Eradication is unlikely in the affected regions and will necessitate growers expand their virus management programs to include this pathogen.

The impact of the NSW detection of CGMMV on domestic markets should be minimal, other than the direct implications for yield at harvest. The other implication for some producers is the impact on international trade as CGMMV-free trading partners may opt to reject shipments from affected areas (DAWE 2019). None of the CGMMV detections reported in this study occurred in close proximity to producers engaged in international trade of cucurbits. NSW is able to continue to export fresh produce safely with the development of Pest Free Places of Production (PFPP; ISPM 10 2016) around key export production regions and ensuring rigorous biosecurity measures are in place for growers with international trade linkages.

Global spread of CGMMV has largely been attributed to the commercial distribution of infected seed (Li et al. 2016). This places Australia in a particularly vulnerable situation as virtually all commercial cucurbit seed is imported (Tesoriero et al. 2016), although commercial seed is tested pre- or post-border for CGMMV (DAWE 2019). There is no known insect vector for CGMMV, however honeybees have been implicated in potentially transporting infected pollen on a local scale (Liu et al. 2014; Darzi et al. 2018). NSW melon crops are routinely serviced by apiarists for pollination (Keogh et al. 2010). Therefore, more work is needed to understand how long viable CGMMV may persist within the hive and if moving hives between farms throughout the season may permit greater spread of infection.

Significantly, our survey generated the first record of WCLaV-1 in Australia (Mulholland et al. 2022). This virus was only reported for the first time in China in 2017 and very little is known about its epidemiology (Xin et al. 2017). This virus is composed of a bipartite genome and belongs to a novel taxon in the family *Phenuiviridae*, tentatively named a member of the genus *Coguvirus* (Zhang et al. 2021). It is unclear how long this virus has been present or how it came to be in Australia. It is possible that the virus is seed borne. We suspect that this transmission pathway is increasingly likely, given that it was recently detected in the United States (Hernandez et al. 2021; Hendricks et al. 2021) and Brazil (Maeda et al. 2021). The sudden WCLaV-1 global range expansion indicates that this virus may be an emerging pathogen for the melon industry. Greenhouse bioassays confirmed that the virus can be mechanically transmitted to watermelon. Successful mechanical transmission was not achieved with other cucurbit species, but this may be a function of sample size (Xin et al. 2017). The impact or potential host range for other cucurbit species such as zucchini requires further investigation. In the NSW isolates, a mixed infection was recorded with WMV, and this is the first record of this type of mixed infection. Co-infection with groundnut ringspot virus (GRSV) and WCLaV-1 were recorded in Brazil (Maeda et al. 2021). Symptoms within the Australian production system seem to be less severe than reported in overseas however the true impact on cucurbit production is yet to be resolved. Further investigation is required to properly assess the epidemiology of this virus in Australia.

Cucurbit viruses were detected in several weed species that were sampled in close proximity to affected cucurbit host crops. Internationally, alternative host plant species from over 28 different plant families have been identified as hosts of either ZYMV, PRSV or WMV. WMV has been reported in over 170 plant species and the host range for CMV is over 1,000 species (Lecoq and Desbiez 2012). Currently, there are 29 known host plant species including 16 cucurbit species for CGMMV (Dombrovsky et al. 2017). From these known host lists, weed hosts of concern for NSW cucurbit production regions include *Solanum nigrum*, *Portulaca oleracea*, *Sonchus oleraceus* and various *Amaranth spp*. These weed species were prevalent during the surveillance in NSW vegetable production regions and have the potential to act as hosts for a variety of viral pathogens. Approximately 30% of the *Araujia sericifera* samples collected in the Sydney Basin were infected with CMV. CMV has a reported host range of over 1,000 species, but currently moth vine only has a single record of being a CMV host in Italy (Yoon et al. 2019). Therefore, we believe our results constitute a host range expansion for this virus in Australia and confirm the Italian record that moth vine can host this virus. This weed is long-lived, highly fecund and very prevalent in the peri-urban areas of the Sydney Basin, Central Coast and Hunter regions and may be acting as an important reservoir for CMV. Additionally, CMV was detected in non-cucurbit vegetable crop species such as bean. Alternative plant hosts can provide a green bridge within and between fields potentially facilitating both mechanical and insect vectored transmission. Furthermore, these alternative hosts are providing a reservoir for the virus to overwinter in between seasonal summer cucurbit crops (Jones 2004; Desbiez and Lecoq 1997).

Positive in-field identification of viruses is difficult given the large overlap of symptoms and many growers were noted to be misidentifying the viruses affecting their crops, attributing symptoms to fungal or bacterial pathogens or nutritional issues. Additionally, many growers were not fully aware of the transmission mechanisms, vectors or host range of viruses in their area. This lack of awareness

highlights the need for further extension and education regarding viral disease management. Greater knowledge of virus distribution across production areas was keenly sought by the growers as a function of this survey, to incorporate more effective strategies into their disease management programs. The landscape of virus populations is ever-changing (Lecoq and Desbiez 2012) and therefore we strongly recommend that a regular disease census be undertaken to maintain current knowledge of virus incidence to minimize disease related crop losses.

Effective virus management is difficult to enact if growers do not fully understand the chain of infection or pathogens that they are managing. By cataloguing viral populations in regional production zones growers may be able to select varieties tolerant to one or more of the key viruses for their region. They will also have a greater appreciation of the transmission mechanisms associated with their key viral pathogens. The potyviruses detected during this survey are transmitted by aphid vectors but they, along with viruses such as CGMMV may also be mechanically transmitted or even vertically transmitted in seed (Simmons et al. 2013; Coutts and Jones 2005; Dombrovsky et al. 2017). Some growers are experimenting with companion plantings as part of their integrated pest management programs. Growers are also incorporating green manure crops and/or border plantings to improve soil health and provide a physical crop barrier for pest insects respectively. Careful selection of these additional plant groups is required to ensure a high burden of virus reservoirs is not being introduced to the farm (Damicone et al. 2007; Coutts et al. 2011). Increased understanding of virus incidence and distribution will allow growers to design targeted weed control programs to reduce non-crop alternative plant hosts.

Relying on growers to self-report new cases of pathogens such as CGMMV relies on industry having adequate knowledge of the symptoms and etiology of the disease. We observed relatively limited industry knowledge about viruses affecting cucurbit production across all regions surveyed. The relative lack of knowledge of virus symptoms and pathology may prevent or at least delay new suspect infections being reported to authorities for diagnosis. Continuing to educate and engage with industry, and where necessary developing material such as factsheets in other languages to cater for growers of non-English speaking backgrounds, will ultimately contribute to better disease detection rates and more effective management approaches. It is vital that government biosecurity agencies work with agronomists and industry leaders that are in a trusted position within communities to improve trust and communication. Regular surveillance activities, undertaken by trained pathologists, are important for recognizing new disease outbreaks, as highlighted by the number of CGMMV detections (50% of NSW detections) from formal targeted surveys.

Acknowledgements This research was conducted as part of the *Area Wide Management of Vegetable Diseases* project funded by Hort Innovation, using the Hort Innovation vegetable research and development levy, with co-investment from NSW DPI and contributions from the Australian Government. The assistance of the Agriculture Victoria Research laboratories is acknowledged with thanks for supporting next generation sequencing work. Dr Bernie Dominiak, Dr David Guest and Dr Nerida Donovan reviewed and improved an earlier version of this manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Altschul S, Madden T, Schäffer A, Zhang J, Zhang Z, Miller W, Lipman D (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402

Australian Plant Pest Database (APPD) (2019) http://www.appd.ala.org.au, accessed 23.11.2019.

Bankevich A, Nurk S, Antipov D, Gurevich A, Dvorkin M, Kulikov A, Lesin V, Nikolenko S, Pham S, Prjibelski A (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput 19(5):455–477

Berendsen S, Oosterhof J (2015) TaqMan assays designed on the Coding sequence of the Movement protein of Cucumber green mottle mosaic virus for its detection in Cucurbit Seeds. Phytopathol 105(11):14–15

BOM (2022) Bureau of Meteorology, http://www.bom.gov.au/climate/drought/archive.shtml, accessed 14.04.2022

Choi S, Choi J, Park W, Ryu K (1999) RT-PCR detection and identification of three species of cucumoviruses with a genus-specific single pair of primers. J Virol 83(1–2):67–73

Choi G, Kim J, Kim J (2004) Soil transmission of Cucumber green mottle mosaic virus and its control measures in watermelon. Res Plant Dis 10:44–47

Clause HM (2015) Vegetable seeds for professionals [Catalogue]. Clause Vegetable Seeds

Contangelo P, Camele I, Malinconico P, & Rana G (1994) Epidemiological studies and control trials against CMV in Basilicata. Inf Agrar 50(29):49–55

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

Coutts B, Kehoe M, Jones R (2011) Minimizing losses caused by Zucchini yellow mosaic virus in vegetable cucurbit crops in tropical, subtropical and Mediterranean environments through cultural methods and host resistance. Virus Res 159(2):141–160

Damicone J, Edelson J, Sherwood J, Myers L, Motes J (2007) Effects of border crops and intercrops on control of cucurbit virus diseases. Plant Dis 91:509–516

Darzi E, Smith E, Shargil D, Lachman O, Ganot L, Dombrovsky A (2018) The honeybee *Apis mellifera* contributes to *Cucumber green mottle mosaic virus* spread via pollination. Plant Pathol 67:244–251

DAWE (2019) Pest Risk Analysis for Cucumber green mottle mosaic virus (CGMMV), Department of Agriculture,

Water and the Environment. Accessed 2 September 2020. www.agriculture.gov.au/biosecurity/risk-analysis/plant/cucumber-green-mottle-mosaic-virus
Desbiez C, Lecoq H (1997) Review: Zucchini yellow mosaic virus. Plant Pathol 46:809–829
Dombrovsky A, Tran-Nguyen L, Jones R (2017) Cucumber green mottle mosaic virus: rapidly increasing global distribution, etiology, epidemiology and management. Annu Rev Phytopathol 55:231–256
EPPO (2015) European and Mediterranean Plant Protection Organization: PM 7/125 (1) ELISA tests for viruses. EPPO Bull 45(3):445–449
Gibbs A, Mackenzie A (1997) A primer pair for amplifying part of the genome of all potyvirids by RT- PCR. J Virol 63:9–16
Gibbs A, Mackenzie A, Wei K, Gibbs M (2008) The potyviruses of Australia. Arch Virol 153:1411–1420
Harper S, Adiyoga W, Subandiyah S, Hidayat A, Gunadi N, Wibowo A, Sulistyaningsih E, Basuki R, Gunaeni N, Thomas J, Menzies N, Kenyon L, Kumar S, Hamill S, Gambley C, Persley D, McGrath D, Li M, Hall Z (2019) Final Report: Sustainable productivity improvements in allium and solanaceous vegetable crops in Indonesia and sub-tropical Australia. Australian Centre for International Agricultural Research. http://www.aciar.gov.au/sites/default/files/project-page-docs/final_report_smcn-2009-056_0.pdf
Hendricks K, Hernandez R, Roberts P, Isakeit T, Alabi O (2021) First report of watermelon crinkle leaf associated virus 1 (WCLaV-1) and WCLaV-2 in watermelon (*Citrullus lanatus*) plants coinfected with Cucurbit chlorotic yellows virus in Florida. Plant Dis. https://doi.org/10.1094/PDIS-06-21-1141-PDN. Online ahead of print. PMID: 34191533
Hernandez R, Isakeit T, Rwahnih M, Villegas C, Alabi O (2021) First report of watermelon crinkle leaf-associated virus 1 (WCLaV-1) and WCLaV-2 infecting watermelon (*Citrullus lanatus*) in the United States. Plant Dis, 105 (7), 2025. https://doi.org/10.1094/PDIS-02-21-0249-PDN
Horticulture Innovation Australia Limited (2020) Australian Horticulture Statistics Handbook: Vegetables 2019/20. Horticulture Innovation Australian Limited 2020
Hongyun C, Wenjun Z, Qinsheng G, Qing C, Shiming L, Shuifang Z (2008) Real time TaqMan RT-PCR assay for the detection of Cucumber green mottle mosaic virus. J Virol 149(2):326–329
ICTV (2022) International committee on taxonomy of viruses, Master species list 2021, V2, https://ictv.global/msl (accessed 15 Sep 2022)
ISPM 10 (2016) International Standard for Phytosanitary Measures, requirements for the establishment of pest free places of production and pest free production sites. Int Plant Prot Convention, https://www.ippc.int/static/media/files/publication/en/2017/03/ISPM_10_1999_En_2015-12-22_PostCPM10_InkAmReformatted.pdf
Jones R (2004) Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res 100:5–30
Keogh R, Robinson A, Mullins I (2010) Pollination Aware: The Real Value of Pollination in Australia. Report prepared for Rural Industries Research and Development Corporation, RIRDC Publication No 10/081
Krueger F (2012) Trim Galore! Available online at: www.bioinformatics.babraham.ac.uk/projects/trim_galore
Langeveld S, Dore J, Memelink J, Derks A, van der Vlugt C, Asjes C, Bol J (1991) Identification of potyviruses using the polymerase chain reaction with degenerate primers. J Gen Virol 72:1531–1541
Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean Region: an ever-changing picture. Adv Virus Res 84:67–126
Lecoq H, Wisler G, Pitrat M (1998) Cucurbit viruses: the classics and the emerging. Cucurbitaceae '98: evaluation and enhancement of Cucurbit germplasm : 30 November-4 December 1998, Pacific Grove, Calif. / James D. McCreight, editor
Li J, Liu S, Gu Q (2016) Transmission efficiency of Cucumber green mottle mosaic virus via seeds, soil, pruning and irrigation water. J Phytopathol 164:300–309. https://doi.org/10.1111/jph.12457
Ling KS, Li R, Zhang W (2014) First report of Cucumber green mottle mosaic virus infecting greenhouse cucumber in Canada. Plant Dis 98:701. https://doi.org/10.1094/PDIS-09-13-0996-PDN
Liu H, Luo L, Li J, Liu P, Chen X, Hao J (2014) Pollen and seed transmission of Cucumber green mottle mosaic virus in cucumber. Plant Pathol 63:72–77
Maeda M, Koyama L, Campos R, Kauffmann C, Osse de Souza J, Gilbertson R, Inoue-Nagata A, Freitas D, Nogueira D, Melo F, Nagata T (2021) First report of watermelon crinkle leaf-associated virus 1 and 2 infecting watermelon (*Citrullus lanatus*) plants in Brazil. Plant Dis. https://doi.org.10.1094/PDIS-06-21-1325-PDN. Online ahead of print. PMID: 34455800
Marino A, Garrido M, Borges O, Gonzalez A (2010) Identification of a viral disease that affects maize in Villa de Cura, Aragua State, Venezuela. Fitopatología Venez 23(1):22–27
Mulholland S, Wildman O, Kinoti W, Constable F, Daly A, Tesoriero L, Maina S, Chapman T (2022) First report of watermelon crinkle leaf associated virus-1 (WCLaV-1) in watermelon (*Citrullus lanatus*) in Australia. J Plant Pathol. https://doi.org/10.1007/s42161-022-01250-8
Park J, Jang T, Song S, Choi H, Ko S (2010) Studies on the soil transmission of CGMMV and its control with crop rotation. Korean J Pesticide Sci 14:473–477
Persley D (2012) Integrated viral disease management in vegetable crops. Horticulture Australia Limited. Final Report VG07128
Pilkington L (2011) Regional extension strategy for managing western flower thrips and tomato spotted wilt virus in the Sydney Region. Horticulture Australia Limited. Final Report VG03098
Reingold V, Lachman O, Blaosov E, Dombrovsky A (2015) Seed disinfection treatments do not sufficiently eliminate the infectivity of Cucumber green mottle mosaic virus (CGMMV) on cucurbit seeds. Plant Pathol 62(2):245–255
Shargil D, Zemach H, Belausov E, Lachman O, Luria N, Molad O, Smith E, Kamenetsky R, Dombrovsky A (2019) Insights into the maternal pathway for Cucumber green mottle mosaic virus infection of cucurbit seeds. Protoplasma 256:1109–1118. https://doi.org/10.1007/s00709-019-01370-6
Sharma A, Katoch V, Rana C (2016) Important Diseases of Cucurbitaceous crops and their management. Chapter 18, handbook of Cucurbits. Growth, Cultural Practices and Physiology
Simmons H, Dunham J, Zinn K, Munkvold G, Holmes E, Stephenson A (2013) Zucchini yellow mosaic virus (ZYMV, Potyvirus): Vertical transmission, seed infection and cryptic infections. Virus Res 176:259–264
Tesoriero L, Chambers G, Srivastava M, Smith S, Conde B, Tran-Nguyen L (2016) First report of Cucumber green mottle mosaic virus in Australia. Australas Plant Dis Notes 11:1. https://doi.org/10.0007/s13314-015-0186-x
Tessitori M, Reina A, Catara V, Polizzi G (2002) *Polygala myrtifolia* as a new natural host of Cucumber mosaic virus. Eur J Plant Pathol 86(12):1403
Tzanetakis I, Martin R (2004) First Report of Beet pseudo yellows virus in Blackberry in the United States. Plant Dis 88(2):223. https://doi.org/10.1094/PDIS.2004.88.2.223C
Ward C, Shukla D (1991) Taxonomy of potyviruses: current problems and some solutions. Intervirology 32(5):269–296
Xin M, Cao M, Liu W, Ren Y, Zhou X, Wang X (2017) Two negative-strand RNA viruses identified in watermelon represent a novel

clade in the Order Bunyavirales. Front Microbiol 8:1514. https://doi.org/10.3389/fmicb.2017.01514

Yoon J, Palukaitis P, Choi S (2019) Host range. In: Palukaitis P, Garcia-Arenal F (eds) Cucumber mosaic virus. American Phytopathological Society Press, pp 15–18

Zhang S, Tian X, Navarro B, Serio F, Cao M (2021) Watermelon crinkle leaf-associated virus 1 and Watermelon crinkle leaf-associated virus 2 have a bipartite genome with molecular signatures typical of the members of the genus Coguvirus (family Phenuiviridae). Arch Virol 166:2829–2834