Detection in Australia of *Cucumber green mottle mosaic virus* in seed lots of cucurbit crops

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Abstract

Cucumber green mottle mosaic virus (CGMMV) was detected in samples of cucurbit seed lots intended for entry into Australia for purposes of sowing. CGMMV was detected in 22 of 631 (3.5%) samples tested in 2016; the virus was detected in seeds of cucumber (8 of 102 samples; 7.8%), melon (13 of 393; 3.3%) and watermelon (1 of 57; 1.8%). Virus was found in seed lots identified as originating from Europe, the Middle East, Africa and North, Central and South America. Exclusion of CGMMV from national entry is an important element of Australia's program of official control for the virus.

Keywords Seed-borne virus · Cucurbitaceae · Official control

The Australian cucurbit-growing industry is dependent on overseas supplies of seed for crop establishment, and large volumes of seed are imported each year. In 2016 the combined weight of the seven largest imported consignments of cucumber seed was approximately 1650 kg, which equates to around 60 million seeds.

The cucurbit-infecting *Cucumber green mottle mosaic virus* (CGMMV), genus *Tobamovirus*, was first described in England in 1935, and spread across the world in the period 1986–2016; it is now considered to be a major threat to cucurbit industries globally (Dombrovsky et al. 2017). The virus is readily transmissible by mechanical means, as well as by insect pollinators (eg honeybees; Darzi et al. 2017) and by contaminated seed derived from infected plants (Liu et al. 2014).

The virus was first detected in the Northern Territory of Australia in 2014 in field crops of watermelon (Tesoriero et

al. 2016), and further outbreaks have been recorded in cucurbit crops on several subsequent occasions. The origin(s) of these infections is considered likely to have been imported virus-contaminated seed (Government of Queensland 2015).

CGMMV is a 'quarantine pest' for Australia, and is under 'official control' (see the International Standard for Phytosanitary Measures No 5 [FAO 2016] for definitions of terms). Following its on-shore detection, Australia imposed 'emergency measures' on importations of seeds of several cucurbit species intended for sowing. By late 2015 the measures had been extended to cover eight species (and their hybrids), including cucumber (*Cucumis sativus*), melon (*Cucumis melo*), squash (*Cucurbita moschata*), pumpkin (*Cucurbita maxima*), and watermelon (*Citrullus lanatus*) (Australian Government Department of Agriculture and Water Resources [DAWR] 2017).

The Australian emergency measures required that all seed of listed species proposed for import be tested for CGMMV using an International Seed Testing Association-accredited ELISA protocol on samples of 9400 seeds from 'large seed lots' (as defined by weight for each species; see DAWR, 2017), or a sample of 20% of the seed lot in the case of 'small seed lots'. To improve the sensitivity of affordable tests, seeds sampled from each seed lot were required to be divided into batches of 100 seeds, with each batch then separately subjected to testing by ELISA. A seed lot was deemed to fail an ELISA CGMMV test if any of its 100-seed batches tested positive; the overall prevalence of the virus in a seed lot (that is, the inferred percentage of individual seeds that would test positive) was estimated from the number of batches that tested positive. Prevalence was estimated using the WinBUGS

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statistical program, assuming all seed from a seed lot had equal probability of being sampled and of testing positive for CGMMV if infected.

Until late 2017, samples from large seed lots were permitted to be tested off-shore or on-shore in Australian Government accredited laboratories, but samples from small seed lots were required to be tested in Australia. Since late 2017 off-shore and on-shore testing has been permitted for both large and small seed lots.

In the calendar year 2016 more than 700 seed lots submitted by 13 commercial seed supply companies were tested by Australian laboratories (Elizabeth Macarthur Agricultural Institute [EMAI], New South Wales, and the Centre for AgriBioscience [AgriBio], Victoria). We report here results of tests on 631 seed lots of five cucurbit species (Table 1); 72 tests were on large seed lots and 559 tests were on small seed lots.

CGMMV-contaminated seeds were detected by both laboratories. As shown in Table 1, positive tests were returned from both large and small lots of imported cucumber and melon seed, and from a single small seed lot of watermelon seed.

In the case of large seed lots, one of 16 seed lots (6.3%) of cucumber seed tested positive for CGMMV, as did three of 19 seed lots (15.8%) of melon. Estimated prevalence of CGMMV in the four positive large seedlots ranged from 0.044 to 0.254%, the latter being recorded from a sample of melon seed of South American origin. In the case of small seed lots, 7 of 86 seed lots (8.1%) of cucumber were found to be contaminated with CGMMV, as were 10 of 374 (2.7%) small seed lots of melon. CGMMV was also detected in one of 54 (1.9%) small seed lots of watermelon.

Neither laboratory detected CGMMV in either large or small seed lots of pumpkin (total $n = 45$) or squash ($n = 34$) in 2016. We note however that in late 2015 the virus was detected by ELISA at EMAI in a single 100-seed batch from

a large seed lot of squash (9400 sampled seeds), and in early 2017 in the single 100-seed batch that was tested from a small seed lot of pumpkin.

Using a hypergeometric distribution, non-detection of CGMMV in a required sample of 9400 seeds from large seed lots (tested as 94 batches each of 100 seeds) indicates, with a probability of 0.99, that the prevalence of the virus is no higher than 0.0439%. The required sample of 20% of a small seed lot provides lower levels of sensitivity for prevalence compared to that for large seed lots, with statistical confidence varying with the number of seeds tested. For example, a negative test result for a seed lot of 30,000 seeds (for which 6000 seeds must be tested, as 60 batches of 100 seeds) provides a probability of 0.99 that the prevalence of the virus in that seed lot is no higher than 0.0689%. This level of detection confidence is close to that achieved for tests on large seed lots. In contrast, a negative result for a seed lot of 500 seeds (from which a single batch of 100 seeds is tested) provides a probability of 0.99 that the prevalence of the virus in the seed lot is no higher than 4.03%, and a probability of 0.95 that prevalence is no higher than 2.65%. Thus, given equal prevalence, contaminated small seed lots have a lower probability of detection than larger seed lots. It is notable that for the testing program reported here the majority of samples analysed (356 of the total of 559 small seed lot samples; 63.7%) were single test batches of 100 seeds.

Rates of virus prevalence in the smallest seed lots are difficult to estimate. While a small number of positive 100-seed batches among a relatively large total number (e.g., among 60 batches) allows an estimate of reasonable accuracy, a positive result in a single batch of 100 seeds taken from a seed lot of 500 cannot be distinguished from an overall prevalence of 100%, although it is intuitively unlikely that this level of prevalence would be the case. Where estimates of virus prevalence

Table 1 Incidence of *Cucumber green mottle mosaic virus* in cucurbit seeds intended for import into Australia for sowing, 2016

	Large seedlots			Small seedlots		
	Number of lots sampled & tested ^a	Number positive	% positive	Number of lots sampled & tested ^b	Number positive	% positive
Cucumber	16	1	6.3	86	7	8.1
Melon	19	3	15.8	374	10	2.7
Pumpkin	20	0	0.0	25	0	0.0
Squash	14	0	0.0	20	0	0.0
Watermelon	3	0	0.0	54	1	1.9
Totals	72	4		559	18	

^a Each tested sample from large seed lots comprised 9400 seeds. ^b Tested samples from small seed lots varied from 100 to 9200 seeds; each tested sample comprised 20% by number of the total seed lot. See text for further discussion

in small seed lot samples were possible, estimates ranged from 0.387 to 1.62%, the latter in a sample of melon seed of Middle Eastern origin.

Consistent with the now apparently global distribution of CGMMV (Dombrovsky et al. 2017), contaminated seed was found in samples from seed lots identified as originating from Europe, the Middle East, Africa, and North, Central and South America.

It is acknowledged that rates of CGMMV infection in seedlings germinated from contaminated seed lots are likely to be lower than the estimated prevalence in the parental seed. Nevertheless, seed to seedling transmission of CGMMV has been repeatedly demonstrated in cucurbit species including cucumber, melon and watermelon (Dombrovsky et al. 2017), and it is reasonable to anticipate similar transmission in the seed lots intercepted in the course of this work.

The results reported here provide an evidentiary base for maintenance of mandatory testing measures in order to ensure that imported cucurbit seeds intended for sowing carry lowest practicable levels of CGMMV contamination. Continuation of such measures has recently been confirmed by the Australian Government Department of Agriculture and Water Resources (DAWR 2017), and is an important aspect of Australia's maintenance of official control of the virus.

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