

## Review Article

# Alarm on the Rapid Increase in Distribution of *Cucumber Green Mottle Mosaic Virus* in China

Jiaying Wang<sup>1</sup>, Wen Li<sup>2</sup>, Jihong Zhang<sup>1</sup>, Ying Xu<sup>1</sup>, Xianfeng Chen<sup>1,\*</sup>

<sup>1</sup>Technical Center, Ningbo Academy of Inspection and Quarantine, Ningbo, China

<sup>2</sup>Key Laboratory of Plant Development, Ningbo City College of Vocational Technology, Ningbo, China

### Email address:

691062809@qq.com (Jiaying Wang), 1203064973@qq.com (Jihong Zhang), Xuy@nbciq.gov.cn (Ying Xu), Chenxf@nbciq.gov.cn (Xianfeng Chen)

\*Corresponding author

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**Abstract:** *Cucumber green mottle mosaic virus* (CGMMV) is becoming a major threat to cucurbit crop production and so listed as the quarantine pathogen in many countries. China, as a large crop producing country, can suffer considerable economic loss from virus dissemination and infection. On account of this situation, our paper lies attention on: a. temporal viral distribution, risks and trend of CGMMV in China; b. possible reasons for rapid dissemination; c. available measures for virus control, especially those in phytosanitary category. In order to limit CGMMV dissemination, quarantine of host plant tissues or seeds needs to be further intensified before transportation or even flowing between different countries.

**Keywords:** *Cucumber Green Mottle Mosaic virus*, Cucurbit Crops, Viral Dissemination, Plant Virus Distribution, Phytosanitary

## 1. Introduction

*Cucumber green mottle mosaic virus* (CGMMV) is a member in genus *Tobamovirus*. It forms rod-shaped particles 300×18 nm in size and contains a single-stranded, positive-sense, ~6.4 kb RNA which contains four open reading frames (ORFs 1-4), encoding two viral replicases, one movement protein and one coat protein, respectively. CGMMV can infect cotyledon, testa and endopleura, and concentrates in embryo. It can also achieve systemic infection through phloem in host plant. Infected plants are characterized by stunted growth, mottling and mosaic on leaves, fruit mottling and distortion.

CGMMV is considered a major threat to cucurbit crop production and listed as the quarantine pathogen in many countries including China. Plant viruses in *Tobamovirus* can cause severe diseases and considerable economic losses on important crop species (e.g., Cucurbitaceae and Solanaceae) worldwide. By 2015, members in *Tobamovirus* had risen to 35, among which CGMMV is the most economically significant. CGMMV has been reported to cause both marketable yield

losses from poor fruit quality and gross yield losses, e.g., 15% and > 50% in cucumber [1] and watermelon [2], respectively. Recently, CGMMV has been found to broaden its affecting areas in both domestic and oversea crop productions. The cucurbit crop production in China is quite huge. Once spread, the virus can have a considerable effect on both agriculture and economy in China.

In this study, we for the first time describe CGMMV distribution in China. This paper focuses on: a. viral distribution, risks and trend in China; b. possible reasons for rapid dissemination globally of CGMMV; c. available measures for virus control, especially those in phytosanitary category. The rapid spreading trend of CGMMV must be stopped so as not to bring huge damage to cucurbit crop production in China and worldwide.

## 2. Distribution of CGMMV in China

CGMMV was first reported on cucumber (*Cucumis sativus*) in 1935, in England [3]. Its distribution all over the world increased

gently from 1935 to 1985, accelerated from 1986 to 2006, while achieved high-speed spread manner over the past decade (2007-2018) [4]. As the largest vegetable producing country, China harvested over 54 billion kg cucumbers in 2013, occupying 76% of total yield all over the world [5]. In China, CGMMV is an alien invader and was first discovered in 2003 [6]. Watermelon samples collected in different regions during 2006-2007 were tested to show an increasing tendency in the distribution pattern of CGMMV in China [7]. So far, CGMMV has been detected and reported in 23 provinces [Table 1] [8]. In 2005, an outbreak of CGMMV emerged on watermelons in Liaoning province, resulting in nearly 333 ha damaged planting

area [9]. Recently researchers sampled muskmelon (*Cucumis melo*), watermelon (*Citrullus lanatus*) and bottle gourd (*Lagenaria siceraria* var. *hispida*) from Zhejiang Province and Shanghai, and confirmed that CGMMV exists readily in those planting areas [10]. In Hunan Province, field survey was carried out on *Myzus persicae* which turned out to be the potential vector for CGMMV [11]. What's more, the pest risk analysis results showed that CGMMV, with a wide range of suitable hosts, high vitality and many ways of transmission, had the risk assessment value at 2.39. In one word, there is high possibility of invasion and diffusion for CGMMV in our country. The corresponding risk management should be done to CGMMV [12].

**Table 1.** The occurrence of CGMMV in China (cited in 8).

Province and region	Disease incidence (or Area of disease occurrence)/ Yield loss
Liaoning	333 hm <sup>2</sup> /13 hm <sup>2</sup>
Jilin	100%/56.49%
Hubei	13.2 hm <sup>2</sup> /-
Zhejiang	70.3 hm <sup>2</sup> /30%
Shandong	87.5%/-
Shanghai	> 50%/-
Jiangsu	40 hm <sup>2</sup> /0.67 hm <sup>2</sup>
Anhui	20 hm <sup>2</sup> /50%
Taiwan, Guangxi, Jiangxi, Beijing, Sichuan, Yunnan, Henan, Guangdong, Hunan, Xinjiang, Gansu, Shaanxi, Shanxi, Hainan, Hebei	-/-

**Table 2.** Natural hosts of CGMMV (cited in 4).

Subdivision	Family	Species	Common name	Crop /weed	Seed transmission	
Asterids	Apiaceae	<i>Heracleum moellendorffii</i>	Eosuri	Weed	Unknown	
	Boraginaceae	<i>Heliotropium europaeum</i>	Common heliotrope	Weed	Unknown	
	Lamiaceae	<i>Moluccella laevis</i>	Bells of Ireland	Weed	Unknown	
	Solanaceae	<i>Solanum nigrum</i>	Black nightshade	Weed	Unknown	
		<i>Withania somnifera</i>	Indian ginseng	Weed	Unknown	
<i>Amaranthus blitoides</i>		Prostate amaranth	Weed	Unknown		
Caryophyllids	Amaranthaceae	<i>Amaranthus graecizans</i>	Mediterranean amaranth	Weed	Unknown	
		<i>Amaranthus muricatus</i>	Rough fruit amaranth	Weed	Unknown	
		<i>Amaranthus retroflexus</i>	Redroot amaranth	Weed	Unknown	
	Chenopodiaceae	<i>Amaranthus viridis</i>	Green amaranth	Weed	Unknown	
		<i>Chenopodium album</i>	Fat hen	Weed	Unknown	
		Portulacaceae	<i>Portulaca oleracea</i>	Pigweed	Weed	Unknown
	Rosids	Cucurbitaceae	<i>Benincasa hispida</i>	White gourd	Crop	Unknown
			<i>Citrullus colocynthis</i>	Bitter apple	Weed	Unknown
			<i>Citrullus lanatus</i>	Watermelon	Crop	Yes
			<i>Cucumis anguria</i>	West Indian gherkin	Crop	Unknown
<i>Cucumis melo</i>			Melon	Crop	Yes	
<i>Cucumis sativus</i>			Cucumber	Crop	Yes	
<i>Cucurbita maxima</i>			Pumpkin	Crop	Yes	
<i>Cucurbita moscbata</i>			Pumpkin	Crop	Yes	
<i>Cucurbita pepo</i>			Zucchini	Crop	Yes	
<i>Echallium elaterium</i>			Squirting cucumber	Weed	Unknown	
<i>Lagenaria siceraria</i>			Bottle gourd	Crop	Yes	
<i>Luffa acutangula</i>			Ridged gourd	Crop	Unknown	
<i>Luffa cylindrica</i>			Smooth loofah gourd	Crop	Unknown	
<i>Momordica charantia</i>			Bitter gourd	Crop	Unknown	
<i>Mukia maderaspatana</i>			Headache bryony vine	Weed	Unknown	
Euphorbiaceae	<i>Trichosanthes cucumerina</i>	Snake gourd	Crop	Yes		
	<i>Chrozophora tinctoria</i>	Turnsole	Weed	Unknown		

### 3. Influence Factors for Rapid Spread

#### 3.1. Expanding Host Range

In previous literature, CGMMV is mostly characterized by a narrow range of plant hosts, infecting primarily the family of

Cucurbitaceae. Recently, CGMMV has been proved to broaden its host range outside the Cucurbitaceae. So far, 16 weed species located in different continents have been identified as natural plant hosts of CGMMV [Table 2][13]. Then here comes the question whether evolution of CGMMV host range represents fuzzy drift or strict jumps [Figure. 1].

CGMMV spread to Euphorbiaceae species in the rosid order represents fuzzy drift. By contrast, CGMMV spread to species in the asterid and caryophyllid orders represents strict jumps [14].

It is hard to recognize CGMMV infection morphologically. In infected foliage and fruit, symptoms vary among different cucurbit species or cultivars of the same species. What's more, symptom development in CGMMV-infected plants is also affected by environmental conditions, plant growth stage at the time of infection, and viral strain. As for weeds, the situation is even worse. It has been reported that virus infection of weeds is more widespread than was commonly thought [14-16]. Symptoms of virus infection are fuzzy to tell and are usually masked by environmental stresses or pest

damages. Accordingly, weeds can maintain the tobamovirus inoculum between successive growing cycles of susceptible crops [17]. Symptomless weeds reservoir within and surrounding infected fields needs to be considered as a potential viral source.

Expanding host range definitely increases the risk of virus spread. In order to gain a wider insight into host range, carefully designed researches focused on additional plants in different locations with various climates and terrains are essential. These research results can contribute to expanding rules of host range which is fundamental for viral control. The interaction mode between host plant and virus deserves further exploration for anti-virus variety development.

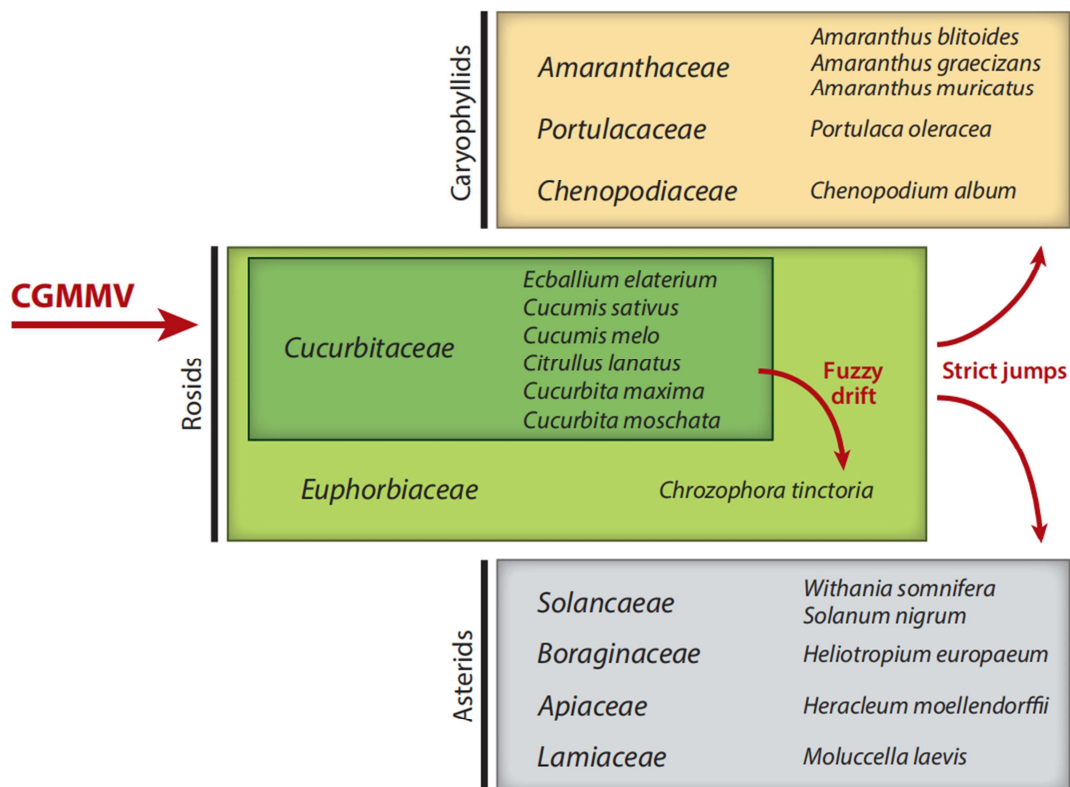


Figure 1. Evolution of CGMMV host range (cited in 4).

### 3.2. Epidemiology

CGMMV achieves its dissemination mainly via two approaches, that is, contact transmission and seed transmission. Tobamoviruses are remarkably stable because of their unique rigid particles, which encapsidate and protect the viral nucleic acid [18]. Attributed to the stable infectious virions, CGMMV can spread readily by contact [19] and by beneficial insects, e.g., bees proved to function in large-scale greenhouse [20] and the potential vector *Myzus persicae* [11]. In principle, asymptomatic CGMMV-infected weeds, contaminated soil, irrigation water, and nutrient solutions can all contribute to contact transmission. On the other hand, seed transmission of CGMMV exists in eight cucurbit crop species [Table 2]. Seeds generated from infected cucurbit plants may also carry virions on the coats. When sown directly in

uninfested soil, the seed transmission rate was higher for watermelon (1-10%), yet low for cucumber and melon (0.1%) [21]. Casual distribution of both CGMMV infected seedlings and contaminated seed stocks constitutes a noticeable way for virus to disseminate into clean cucurbit fields.

CGMMV has such a complex disease cycle [Figure 2] that it can hardly be eliminated once introduced into planting area. One reason for the complex disease cycle is that CGMMV can persist with cucurbit crops absent via (1) contaminated plant debris all over the infested field, any surviving root tissues from infected hosts, and infectious virions in the soil and (2) seed contamination, weed hosts and volunteer cucurbit crops infection, and virions on unsterilized tools, machinery, and other surfaces. Once CGMMV emerged, all infected plants should be safely dealt with, and infested fields be treated

under stringent quarantine and biosecurity restrictions. No CGMMV hosts are allowed in infested farms for at least two years and phytosanitary certification is necessary before any production. Eliminating potential CGMMV reservoirs (alternate host weeds or volunteer cucurbit crops) is crucial to clearing carryovers between successive cucurbit seasons.

A wider perspective of the complex agricultural diseases to find the most suitable disease management measures is in urgent need. Integrated disease management (IDM) including phytosanitary (hygiene), cultural (agronomic), chemical

(against vectors), host resistance, and biological control measures designed for a given virus-host patho-system and specific growing situation can provide the most efficient approaches to control virus disease. Phytosanitary is the fundamental and most important approach to prevent viral introduction and limit further dissemination at the initial stage. Plant quarantine has been guarding the domestic biosecurity, keeping abroad plant pathogens off and guaranteeing the exporting goods' quality for quite a long time.

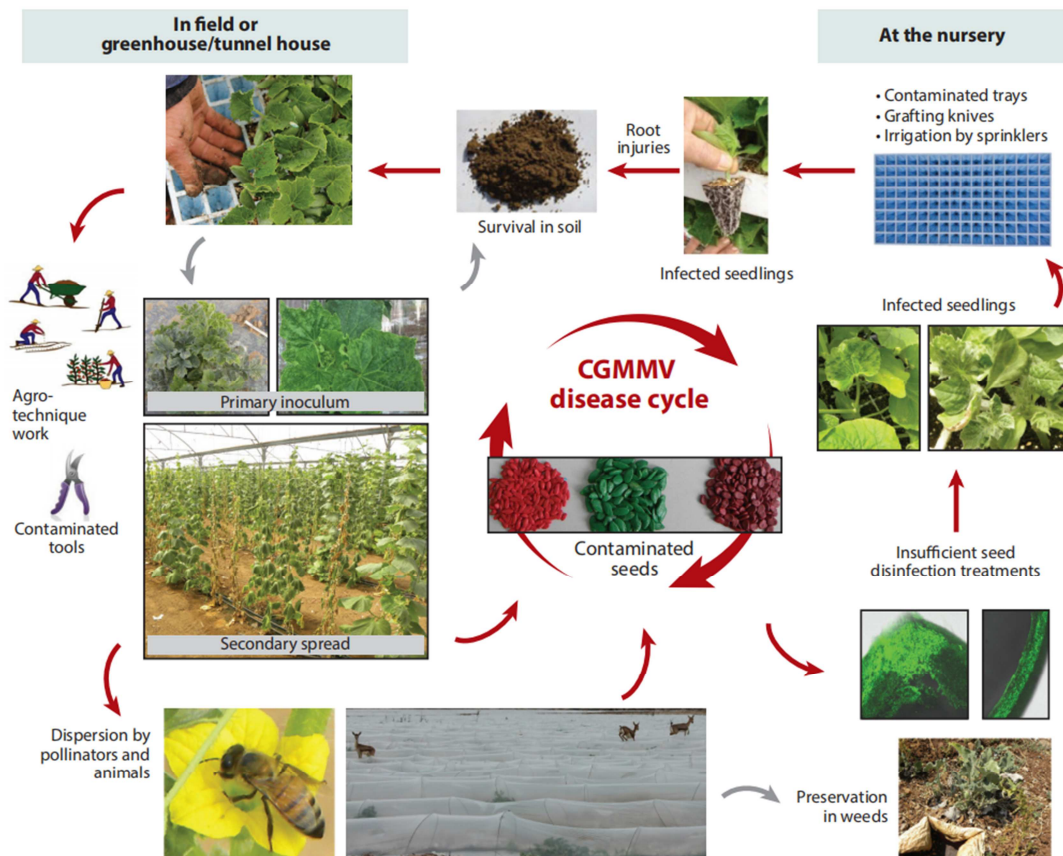


Figure 2. Disease cycle (cited in 4).

### 3.3. The Importance of Phytosanitation

Long-distance spread of economically risky viruses is being facilitated by trade globalization. Such dispersal is taken to the next level by ever increasing agricultural diversification, extensification, and intensification or relaxation of national plant quarantine regulations [22]. As for CGMMV, its distribution worldwide increased rather slowly from 1935 to 2006, while accelerated considerably in the past 11 years (2007-2018). Rapid CGMMV dispersion recently can be explained in a similar way: Cucurbit seed crop production by multinational companies in the Indian subcontinent or Southeast Asia led to seed crop infection, seed contamination, and spread of seed-borne CGMMV by the international seed trade [4]. Since CGMMV spreads over long distances mainly via contaminated cucurbit seeds or infected seedlings, early detection and identification of seed-borne virions are vital

before exporting/importing seed stocks. Phytosanitary measures are quite essential for entry-deterrence of those pathogens that approach long-distance spread through seeds or other kind of propagules. The Australian emergency measures required that all seed of listed species proposed for import should 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' [23].

## 4. Phytosanitary Methods for Virus Control

To prevent virus introduction and further spreading, the early detection and identification are required. Development

of improved routine procedures for large-scale detection in seed, seedling, soil, and water samples will help ensure CGMMV's rapid and accurate diagnosis. Different techniques have been established for detection and diagnosis of CGMMV: RT-PCR [24], real time RT-PCR [25], transmission electron microscopy (TEM), immune capture (IC)-RT-PCR [26], ELISA using polyclonal antibodies (PABs) [27] and monoclonal antibodies (MAbs). RT-LAMP, as a new method, has its advantages of high sensitivity, less time-consuming and more environmentally friendly. RT-LAMP is suitable for rapid virus detection in places without complex machines, like ports. Development of RT-LAMP assays that could be widely applied for detection of a wide range of viral isolates requires the knowledge of gene sequences, careful primer design, and assay optimisation. Taking into account the genetic diversity of the CGMMV population, the primers for an RT-LAMP assay should be designed based on the alignment of CP sequences instead of a single sequence [28, 29].

Reverse transcription recombinase polymerase amplification (RT-RPA), another new method, is time-saving and has a high sensitivity. This developed CGMMV RT-RPA method could be used as a promising diagnostic tool in less equipped laboratories or in the field. CGMMV RT-RPA was 100-fold more sensitive than the normal RT-PCR assay [30]. The application of High-Throughput Sequencing (HTS), also known as next-generation sequencing, has been successful for virus discovery to resolve disease etiology in many agricultural crops. The greatest advantage of HTS over other diagnostic approaches is that it gives a complete view of the viral phytosanitary status. In theory, HTS can detect all viruses in a single assay and performance is limited only by the completeness of the reference database (s) against which the sequences are compared. Sequence information obtained can also be used to provide insight into the virus population structure, ecology or evolution or into virus variants that may contribute differently toward disease etiology. Another advantage of HTS is that sequence data can be analyzed by multiple end-users or may be re-analyzed as databases are expanded. The advancement in HTS technologies undoubtedly brought great potential for virus detection and discovery. However, like any new technology, HTS-based approaches should be validated for sensitivity, specificity, reproducibility and repeatability before their routine implementation [31].

## 5. Conclusion

As an economically important pathogen, CGMMV is receiving more and more attentions regarding its viral characters, epidemiology and management measures. Although CGMMV is widespread all over the world, similar out-break reports in China are few in number. High possibility of invasion and diffusion for CGMMV exists in our country. The corresponding risk management should be done. Based on the expanding host range, virus epidemiology and phytosanitary importance, current attentions should be paid on blocking virus introduction

from abroad and eliminating existing domestic virus as soon as possible. National phytosanitary surveillance must ensure that a representative subsample from every imported seed stock is tested by a suitably accredited laboratory before release. Beside those phytosanitary actions, there are some other methods to control infectious virions. Sodium hypochlorite is the most effective disinfectant against tobamoviruses [32]. Steam heat or ozone treatments and ultraviolet irradiation is also used to destroy infectious virions [33]. Cross-protection is an attractive biological approach for protecting crops against CGMMV. Attenuated strains of CGMMV can be constructed by modifying the viral genome [34]. Studies on the relationship between infectivity and these mutations have received a major impetus from the perspective of CGMMV pathogenesis.

Future researches can focus on: 1) evaluating other means of viral spread, including wind-assisted contact transmission, and whether other beneficial insects have a role in spreading CGMMV via pollination or plant wounding; 2) discoveries on virus genetic diversity and the difference between domestic strains and other strains on infection, host range, pathogenicity, persisting ability in all kinds of intermediate hosts. 3) potential reservoirs in other plants or even insects. 4) the life cycle of CGMMV surviving outside the cucurbit growing season in diverse alternate weed hosts in different world regions and whether carryover occurs in weeds via seed-borne infection.

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