



United States Department of Agriculture

Animal and Plant Health Inspection Service - Plant Protection and  
Quarantine

**Survey and Response Plan for *Tobamovirus*  
*Cucumber green mottle mosaic virus* and  
*Watermelon green mottle mosaic virus*  
in agricultural field settings and post-harvest  
detections in seed**

This response plan provides guidelines and actions necessary to survey and respond to *Tobamovirus Cucumber green mottle mosaic virus* (CGMMV) and *Watermelon green mottle mosaic virus* (WGMMV) in agricultural field settings and post-harvest detections in seed. This plan supplements the information contained in the New Pest Response Guidelines for *Tobamovirus Cucumber green mottle mosaic virus*; it is used in conjunction with other agency regulations, guidelines, and manuals when conducting program activities.

Prepared by CGMMV/WGMMV Cross Functional

Working Group March 8, 2024

## Appendix 2: Sanitation

Protocol for the Sanitation and Disinfection of Tomato Brown Rugose Fruit Virus (ToBRFV) and Cucumber Green Mottle Mosaic Virus (CGMMV) on Equipment and Structures. It is assumed that these disinfection protocols will be effective for all tobamoviruses including WGMMV.

### Sanitation Treatment Follow a two-step process:

#### 1. Pre-cleaning of equipment and structures

- Wastewater should be captured onsite. If wastewater needs to be removed away and discharged, an APHIS-approved sewage system should be used to prevent virus dissemination.
- Remove all litter, including debris and organic matter, from the surfaces to be treated.
- Thoroughly clean and rinse all surfaces to remove soil and debris. The efficacy of disinfectants may be reduced in the presence of organic residues.

#### 2. Surface treatment using disinfectant

- Use one of the chemicals below in Table 1 or any commercial products containing one of the active ingredients based on its availability.

Appendix 2, Table 1. ToBRFV and CGMMV disinfectants and their application (adapted from Chanda et al. 2021)

Disinfectant (active ingredient)*	Application rate
Sodium hypochlorite [NaOCl]	0.53%
Glutaraldehyde [OHC(CH <sub>2</sub> ) <sub>3</sub> CHO, C <sub>5</sub> H <sub>8</sub> O <sub>2</sub> ]	0.22%

\* Commercially-available products, such as Clorox® (Clorox Company, Oakland, CA) or Virocid® (CID Lines N.V., Belgium), can be used as stock to prepare a working solution.

- Each disinfectant should be prepared daily from the stock according to the manufacturer's instructions or EPA label.
- Saturate all surfaces with the appropriate disinfection solution. Wait at least 15 minutes before rinsing. Prepare a working solution using the dilution formula or Table 2

#### 1. Dilution using the formula

The formula is  $C_s \cdot V_s = C_w \cdot V_w$ .

The objective is to find the amount of stock solution required ( $V_s$ ) to produce the desired volume of working solution ( $V_w$ ) at the required concentration ( $C_w$ ) of the active ingredient. The active ingredient concentration in the stock solution is  $C_s$ .

Because you are attempting to find  $V_s$ , the formula can be rewritten as  $V_s = (C_w \cdot V_w) / C_s$ .

For example, when using Clorox® Disinfecting Bleach as the stock solution, the initial concentration ( $C_s$ ) of the active ingredient sodium hypochlorite is 7.5%. In order to determine the amount of Clorox® Disinfecting Bleach needed to make 15 gallons of working solution of sodium hypochlorite disinfectant at a concentration of 0.53%, simply multiply  $C_w * V_w$  and then divide by  $C_s$ . In this example,  $C_w * V_w = 0.53\% * 15\text{g gallons} = 7.95$ . Then divide 7.95 by 7.5%:  $7.95/7.5 = 1.06$  gallons. The volume ( $V_s$ ) of stock solution (Clorox® Disinfecting Bleach) required is 1.06 gallons, which can be mixed with water to a final volume of 15 gallons.

When using the formula, make sure that  $C_s$  and  $C_w$  or  $V_s$  and  $V_w$  are in the same units, respectively. In the above example, the units for  $C_s$  and  $C_w$  were the percentage of the active ingredient, and the units for  $V_s$  and  $V_w$  were in gallons.

## 2. Preparation of a working solution using Table 2

Appendix 2, Table 2. Dilution table to prepare the working solutions (0.53% sodium hypochlorite or 0.22% glutaraldehyde) from the commercial products\*

Commercial Products	1 gal working solution	5 gal working solution	10 gal working solution	15 gal working solution	25 gal working solution
Clorox® Disinfecting Bleach (7.50%)	9.05 fl oz	45.23 fl oz	90.45 fl oz	135.68 fl oz	226.13 fl oz
Clorox® Germicidal Bleach (7.55%)	8.99 fl oz	44.93 fl oz	89.85 fl oz	134.78 fl oz	224.64 fl oz
Clorox® Performance Bleach (7.55%)	8.99 fl oz	44.93 fl oz	89.85 fl oz	134.78 fl oz	224.64 fl oz
Clorox® Commercial Solutions (8.25%)	8.22 fl oz	41.12 fl oz	82.23 fl oz	123.35 fl oz	205.58 fl oz
Virocid® CID LINES (10.725%)	2.63 fl oz	13.13 fl oz	26.26 fl oz	39.38 fl oz	65.64 fl oz

The stock volumes (fl oz) needed for the amounts (gal) of working solutions are listed in Table 2. For example, to prepare 10 gal of working solution (containing 0.53% sodium hypochlorite) from Clorox® Commercial Solutions (8.25% A. I.), aliquot 82.23 fl oz of the stock (in Table 2, check the stock in the first column and 10 gal working solution in the first row, at the cross of the column and row, 82.23 fl oz is the stock amount needed), pour 82.23 fl oz stock into a container with a mark of 10 gal, add water to a total volume of 10 gal, and mix well before use.