Surprising Results from a Search for Effective Disinfectants for *Tobacco mosaic virus*–Contaminated Tools

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ABSTRACT

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Over 100 years after its discovery, *Tobacco mosaic virus* (TMV) remains an economically important pathogen for producers of many vegetatively propagated crops including petunias (*Petunia × hybrida*). To directly address this concern, we have developed a robust system to determine efficacy of disinfectants for treating TMV-contaminated cutting tools using a combination of preliminary screens and replicated trials. Contrary to widely held beliefs, wild-type (wt) TMV and four additional tobamovirus species infected four petunia cultivars without producing obvious viral symptoms. In contrast, a petunia isolate of TMV with 99.0% (nucleotide) and 99.4% (amino acid) coat protein sequence identity to wt TMV produced symptoms on all but one tested cultivar. We also show that TMV transmission can occur up to the twentieth petunia plant cut following a single cutting event on a TMV-infected plant. Although many new products are now available, treatment of TMV-contaminated tools with a 20% (wt/vol) solution of nonfat dry milk (NFDM) plus 0.1% Tween 20 or a 1:10 dilution of household bleach (0.6% sodium hypochlorite), two “old standbys”, completely eliminated TMV transmission to petunias. Treatment of contaminated tools with 1% (wt/vol) Virkon S or 20% NFDM also significantly reduced the incidence of infected petunias. Other treatments identified in the preliminary screens are candidates for the second phase of screening that simulates contamination during the process of taking cuttings.

The wholesale value of floricultural crops for the 15 largest producing U.S. states from growers with at least \$10,000 in sales in 2008 was estimated at over \$4.22 billion; petunias (*Petunia × hybrida*) were ranked third among floricultural crops (in excess of \$120 million) (38). The movement of vegetatively propagated plant material and the continuing introduction of new cultivars and plant species are important routes for introduction of viruses into greenhouse operations. Because of the intensity of production of ornamentals grown at high plant densities, a virus outbreak in a greenhouse can quickly spread and devastate entire crops. Thus, disinfecting tools remains a critical process in pre-

venting virus spread during propagation and production.

Losses in the floriculture industry due to viruses are difficult to quantify because most such events are not widely publicized. In Georgia, virus diseases were responsible for an estimated \$1.79 million in ornamental crop losses in 2006 (41). However, expensive precautionary recalls of plant material in response to the detection of viruses, including *Tobacco mosaic virus* (TMV), in stock plants result in much higher indirect costs. To minimize losses during vegetative propagation, stock plants are routinely indexed for viruses. Major propagators also have strict sanitation protocols to minimize the introduction and spread of pathogens. New viruses and viroids are being found through virus-indexing and testing programs (1,6), and known viruses and viroids continue to be detected in new hosts (7,21,39), emphasizing the importance of increased vigilance and improved sanitation protocols that are based on efficacy data gathered from rigorous testing of available disinfectants.

Of the over 130 viruses known to infect petunias, the most commonly detected in the United States is TMV (29). TMV is the type species of the genus *Tobamovirus* and has a wide host range that is estimated to include over 200 species of plants (34). TMV is transmitted mechanically through contact between infected and uninfected

plants, by workers handling uninfected plants with contaminated hands, or through contaminated tools during propagation (28). TMV virions are extremely stable and are able to survive for months outside the host, such as on greenhouse benches, tools, and surfaces (12). Disinfecting tools is a critical process in preventing this route of virus spread. TMV causes a variety of symptoms on petunia including vein-clearing, mosaic patterns on leaf tissue, flower color break, stunting, necrosis, and leaf rugosity (4,14,15,26,29,37).

A number of disinfectants are used to control plant pathogens and microorganisms on hard surfaces (20). However, only a limited number of products are labeled for use within plant production facilities. The ideal disinfectant would have activity against a broad spectrum of pathogens, and be inexpensive, widely available, and safe for workers, plants, equipment, and the environment. To be practical, a disinfectant for tools must also be effective with a relatively short contact time (1 min), to enable disinfecting one tool while using another. Some of the more commonly used greenhouse disinfectants contain quaternary ammonium chloride salts, hydrogen dioxide, sodium hypochlorite (NaOCl), peroxide compounds, or alcohol. NaOCl and milk are often recommended for disinfection of cutting tools against viruses and other materials such as trisodium phosphate (10,11) or milk for hand sanitation (19,28). Some materials are phytotoxic and cannot be used when plants are present. Ethyl alcohol (70 to 90%) is effective against viruses (22), although it is not practical in greenhouses due to its flammability. Disinfectants produced from halogens are inexpensive and readily available. Chlorine destroys all types of microorganisms and viruses, but is irritating to the skin, corrosive to tools, and diluted solutions have a short half-life. NaOCl has been shown to reduce fungal spore viability on greenhouse hard surfaces, including plastic, wood, and metal (16,17). Tool disinfection trials for management of *Hibiscus latent Fort Pierce virus* (HLFPV), a tobamovirus that infects hibiscus, demonstrated that a 1-min contact time with swimming pool chlorine (10% NaOCl) prevented virus transmission from virus-contaminated knives to uninfected hibiscus plants (23). Infectivity of sap prepared from *Odontoglossum ringspot virus*–

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infected orchids was abolished with a 1:10 dilution of bleach, but not with a 1:50 dilution (22).

Spray treatment with whole or skim milk is recommended to prevent the plant-to-plant transmission of TMV prior to handling of tobacco plants (32). However, skim milk was not effective against *Cymbidium mosaic virus* in orchids (22). Treatment of HLFPV-contaminated knives with 20% (wt/vol) nonfat dry milk (NFD) for 1 min eliminated HLFPV transmission to uninfected hibiscus (23). Milk and whey sprayed on leaves have also been shown to have some efficacy against powdery mildew on cucurbits, grapes, and tomatoes (8,9,18,36).

Trisodium phosphate has been used for years as a hand-washing agent for workers, seed treatment, or to disinfest tools contaminated with plant viruses (10,11). In a study where 0.1, 1, and 3% trisodium phosphate was mixed with sap from TMV-infected plants and then rubbed on an indicator plant, only 3% trisodium phosphate inactivated the virions after 15 min (10). Treatment of seeds contaminated with Tropical soda apple mosaic virus (TSAMV), a tobamovirus that infects a solanaceous weed, with a saturated solution of trisodium phosphate (~17.3% [wt/vol]) for 30 min effectively eliminated detectable TSAMV contamination of seeds, thus preventing seedling infection (2).

The TMV-petunia system is an ideal model system to study greenhouse sanitation in an herbaceous crop because of both its economic importance and the difficulty of eradication. Currently, there are very few published studies directly comparing materials for disinfecting hard surfaces contaminated with plant pathogens (16,17) or disinfecting tools (13,23,31,35). The objective of this research was to establish in two geographically disparate locations (Ohio and Florida) a two-tiered system consisting of a preliminary disinfectant screen followed by replicated trials to test disinfectants against one of the most important viral greenhouse pathogens. This information will help determine the most effective products for disinfecting TMV-contaminated tools during vegetative propagation and other cultural manipulations.

MATERIALS AND METHODS

Petunia sources and culture. *Petunia × hybrida* Sweetunia 'Blue Sky' and Surprise 'White Improved' unrooted cuttings were provided by Dümmer USA and rooted by Timbuk II Farms in Granville, OH in 102-cell plug trays (Dillen Products, Middlefield, OH) for use in experiments in Ohio. Surfinia 'Baby Red' and 'Magenta', Supertunia 'White' and 'Red', and Sanguna 'Midnight Blue' petunia liners were provided by EuroAmerican Propagators, Encinitas, CA in 84-cell liner trays for use in experiments in Florida and Ohio. Plants

were grown in a greenhouse and fertilized with 20-10-20 fertilizer (Peters Professional General Purpose, The Scotts Company, USA). Liners were visually inspected for symptoms, and extra Blue Sky and White Improved petunias were transplanted into 5.5-in. pots and maintained as stock plants. These plants were later tested for TMV infection and found not to be infected. To minimize contamination, new plug trays, pots, and carrying trays were used throughout the study, and benches were disinfected between experiments. Plants were watered by hand by trained staff or from an automatic sprinkler system as needed. Greenhouse air temperatures in Ohio ranged from 17.9 to 36.7°C, and relative humidity ranged from 56.8 to 82.0%. Greenhouse air temperatures in Florida ranged from 18.7 to 34.4°C, and relative humidity ranged from 28 to 89.5%. Insecticides and fungicides were sprayed as needed to maintain low pest and fungal populations.

Virus sources and characterization. In Florida, the tobamoviruses TMV (U1 strain), hereafter referred to as wild-type (wt) TMV; *Tomato mosaic virus* (ToMV; 24); *Pepper mild mottle virus* (PMMoV; 3); *Tobacco mild green mosaic virus* (TMGMV); and TSAMV (2) were used as inoculum. Partially purified wt TMV virions were isolated using a typical tobamovirus protocol (40) and used to contaminate tools. An extinction coefficient of $E_{260nm}^{0.1\%} = 3.00$ was used to estimate virion concentration spectrophotometrically (42).

Petunias exhibiting foliar symptoms or flower color break symptoms suggestive of virus infection were collected in Ohio in 2006. Plants were tested for tobamovirus infection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercially available TMV antiserum that detects several tobamoviruses in addition to TMV (Agdia, Inc., Elkhart, IN). Several tobamovirus isolates were selected for further characterization. Total RNA was independently extracted from leaf tissue of several symptomatic TMV DAS-ELISA-positive petunia cultivars with the RNeasy Plant Mini Kit (Qiagen). First strand cDNA was primed with T-4, a primer complementary to TMV nts 6351-6371 (5'-GGGATTCTGA ACCCCTCGCTTT-3') and synthesized with *Moloney murine leukemia virus* reverse transcriptase at 45°C for 45 min. The coat protein (CP) open reading frame (ORF) was amplified by polymerase chain reaction with primers T-4 and Tob-Uni 2 (27). The resulting ~850 bp amplicons were sequenced at the Plant Microbe Genomics Facility at The Ohio State University. The CP sequences of unique TMV petunia isolates are deposited in GenBank as Accession Nos. GQ370522-GQ370525.

The Ohio petunia tobamovirus CP sequences were subjected to BLASTN and

BLASTX queries to identify the most similar viruses (5). Multiple alignments were made using the CP sequences of four Ohio petunia isolates (pet5, pet7, pet8, and pet9), wt TMV, TMV isolates 1853 and 1373-Le95-208 (27), and sequences of all available TMV isolates from petunia (Ohio-V, TMV-p, and TMV-pet) using ClustalW2 (25).

Petunia inoculations. To find suitable tobamovirus/cultivar combinations for the planned disinfectant studies, one set of liners of four vegetative petunia cultivars (Surfinia 'Baby Red' and 'Magenta', Supertunia 'White', and Sanguna 'Midnight Blue') were transplanted into 4-in. round azalea pots containing Pro-Mix BX (Premier Horticulture) in FL. Several plants of each cultivar were individually mechanically inoculated with a 1-mg/ml solution of TMV virions or a 0.1-mg/ml solution of ToMV or PMMoV virions, or a homogenate of TMGMV-infected tobacco (*Nicotiana tabacum* cv. Xanthi) or TSAMV-infected *N. benthamiana* leaves (1:10 wt/vol) in 20 mM sodium phosphate buffer (pH 7.0) containing 1% (wt/vol) Celite. A second set of petunia liners was transplanted into 3-in. square pots containing Metro-Mix 360 (Sun Gro Horticulture) in Ohio and mechanically inoculated with a homogenate of TMV-pet7-infected petunia leaves in the buffer described above. All plants were rated for local and systemic symptoms for at least 30 days postinoculation. Plants were further tested by DAS-ELISA for all viruses except TSAMV, which was assayed as previously described (2).

Serial inoculations with clippers. The objective was to determine how many petunia plants could be infected from clippers contaminated with a single cut on a virus-infected plant. Each of five grower-supplied, used clippers (Bahco P128-19 harvesting snips, SNA Europe) was used to make two transverse stem cuts on an uninfected Baby Red petunia plant, followed by a single transverse stem cut on a TMV-infected tobacco plant. Next, each TMV-contaminated clipper was used to sequentially make two transverse stem cuts on each of 20 uninfected Baby Red petunia plants, recording the order of cutting. All plants were maintained for 5 weeks and evaluated visually and by DAS-ELISA for TMV infection.

Disinfectants. The following products were obtained for testing as disinfectants against TMV-contaminated blades: (i) household bleach (6% NaOCl), (ii) swimming pool chlorine (10% NaOCl), (iii) ZeroTol (27% hydrogen dioxide, Biosafe Systems LLC, Glastonbury, CT), (iv) Green-Shield (10% *n*-Alkyl [60% C14, 30% C16, 5% C12, 5% C18] dimethyl benzyl ammonium chloride and 10% *n*-Alkyl [68% C12, 32% C14] dimethyl ethylbenzyl ammonium chloride, Whitmire Micro-Gen Research Laboratories, St.

Louis, MO), (v) Virkon S (20.4% potassium peroxymonosulfate and 1.5% sodium chloride, Antec International Ltd., UK), (vi) MENNO Clean (9% benzoic acid, MENNO-Chemie, Germany), (vii) 'TSP' (75 to 80% trisodium phosphate dodecahydrate, 20 to 25% sodium sesquicarbonate, The Savogran Co., Norwood, MA), (viii) 'Tri-Sodium Phosphate (T.S.P.)' (30 to 60% sodium phosphate, tribasic, 10 to 30% sodium carbonate, anhydrous, DAP, Inc., Baltimore, MD), (ix) 'TSP-PF' (90 to 95% sodium sesquicarbonate, 0 to 5% sodium metasilicate, 0 to 5% EDTA, The Savogran Co.), (x) 'TSP Substitute' (anhydrous sodium carbonate, Custom Building Products, Seal Beach, CA), (xi) TwinOxide (currently sold as GO2; 0.3% chlorine dioxide, BTO Solutions, Roseville, CA), (xii) Professional Lysol Brand III Disinfectant Spray (Lysol, 0.1% alkyl [50% C14, 40% C12, 10% C16] dimethylbenzyl ammonium saccharinate, Reckitt Benckiser, Parsippany, NJ), and (xiii) nonfat dry milk (NFDM, Carnation Instant, Nestlé, USA). Products were either tested undiluted, or dilutions were prepared fresh for each experiment with deionized water. The flame treatment was generated by a propane plumber's torch (Florida) or butane laboratory burner (Ohio).

Saturated solutions of 'TSP', Trisodium phosphate (T.S.P.), 'TSP-PF', and 'TSP Substitute' were prepared and tested in the preliminary screens (experiments 1 to 3). Undiluted household bleach (6% NaOCl) was used in experiment 1, whereas swimming pool chlorine (10% NaOCl) was used in experiments 2 and 3. Lysol was sprayed directly on the blades. NFDM was used at 20% (wt/vol) throughout this study. All other materials were initially tested at label rate.

Experiment 1. 84-cell liner trays of Supertunia 'Red' petunias were divided into strips consisting of 21 plants (three rows of seven) in Ohio. New heavy duty (0.012 in.) single edge surgical carbon steel razor blades (American Safety Razor Co., Cedar Knolls, NJ), which were used throughout this study, were contaminated by making two cuts on a petunia stock plant infected with TMV-pet7. Blades were next immersed in 40 ml of fresh disinfectant solution or water in 100 × 15 mm disposable sterile petri dishes for 1 min. Each blade was used to make a single transverse cut to the stem of one single-stemmed uninfected Red petunia liner. The negative control (mock) treatment consisted of cutting petunia liners with clean, untreated razor blades. To minimize cross-contamination between treatments, sections of liner trays were spaced on the bench. Symptomatic plants were randomly sampled and tested for infection by DAS-ELISA as described above. All remaining asymptomatic plants were tested by DAS-ELISA at the termination of the experiment.

Experiments 2 and 3. We were unable to mechanically infect Supertunia 'Red' pe-

tunias with wt TMV. Thus, experiments 2 and 3 used Surfinia 'Baby Red' petunias grown from liners transplanted into 4-in. round azalea pots in Florida. Five new razor blades were stacked into groups and held with 2-in. metal binder clips with the cutting edges oriented away from the clip. Each group of five was contaminated by dipping the blades in a suspension of 1 mg/ml TMV virions for 5 s. Blades were then used directly (no sanitation), or each group of five razor blades was immersed in a beaker containing 200 ml of disinfectant solution for 1 min, followed by a 1-min soak in 200 ml of fresh tap water. Lysol was sprayed on contaminated blades and allowed to remain for 1 min prior to the 1-min water soak. Following 1 min of flame treatment, blades were allowed to cool for 1 min prior to the 1-min water soak. All blades for each treatment were placed in a sterile glass petri plate following the water soak. Each blade was then used to make an average of four (range of three to seven) transverse cuts on the stems of an uninfected Baby Red petunia plant. To minimize cross-contamination, pots were spaced apart from one another. New growth of each plant was sampled at 10 weeks post-cutting (wpc) by DAS-ELISA to determine the number of TMV-infected plants.

Experiment 4. The selection and/or modification of treatments included in experiment 4 was based on results from experiment 1 and input from industry co-operators. Based on low efficacy in experiment 1, some treatments were excluded from experiment 4. Rates of active ingredients for the following treatments were changed: household bleach was diluted 1:10 (0.6% NaOCl); 'TSP' was tested at (3% wt/vol); Green-Shield was tested at twice label rate (2 tsp/qt) for 1 min and at label rate (1 tsp/qt) for 3 min; and NFDM (20% wt/vol) was tested with and without 0.1% Tween 20. ZeroTol (1:100 dilution) and Virkon S (1% wt/vol) were included in experiment 4.

A randomized complete block design was used in experiment 4 with Blue Sky and White Improved petunia liners received in Julian week 13 (trials A and B, respectively) and repeated with Blue Sky and White Improved liners received in Julian week 15 (trials C and D, respectively). Plants were arranged within new liner trays to prevent contact between treatments and to increase air circulation. Treatments consisted of six plants arranged in a row. Each 102-cell (17 × 6) liner tray held five treatments, with three rows of empty cells separating treatments to prevent cross-contamination. Each treatment was replicated five times per cultivar. The percent incidence of TMV infection was calculated for each six-plant replicate.

Groups of six new razor blades were stacked and clipped as described above and placed for 30 s in a 100 × 15 mm dis-

posable sterile petri dish containing a homogenate of fresh TMV-pet7-infected petunia leaves prepared by grinding 5 g of fresh leaf tissue in 35 ml of sterile deionized water in a mortar and pestle. Each set of blades was briefly rinsed in deionized water, gently blotted to remove excess liquid, and immediately immersed in 20 ml of fresh disinfectant solution or water in a 60 × 15 mm disposable sterile petri dish for 1 min, with the exception of the Green-Shield (label rate) for 3 min. Each treated blade was used to make a single transverse cut to the main stem of one uninfected petunia liner. The negative control (mock) treatment consisted of cutting petunia liners with new, untreated razor blades. Liners were maintained in the laboratory overnight at 25°C before being returned to the greenhouse the following morning.

Plants were visually rated for symptom development (flower break and/or leaf mosaic). At 55 days post-cutting (dpc) (Blue Sky) and 60 dpc (White Improved), the newest fully expanded flower and associated terminal bud of each asymptomatic plant and one symptomatic plant per block was removed with a new razor blade. Plants with symptoms consistent with TMV infection that were not sampled were scored as infected based on our preliminary research with these cultivars. Tissue was analyzed by DAS-ELISA as described above.

Following DAS-ELISA, all asymptomatic plants were potted into 3-in. square pots 1 to 2 wpc. When symptoms were visible on all but one DAS-ELISA-positive White Improved petunia plant, all remaining plants were rated for symptoms. Final symptom ratings were made 96 to 98 dpc (Blue Sky) and 97 to 102 dpc (White Improved). None of the asymptomatic plants that originally tested negative by DAS-ELISA developed symptoms. Ten percent of the plants were arbitrarily sampled and retested by DAS-ELISA. None of the original DAS-ELISA-negative plants tested positive on the second DAS-ELISA. Percent incidence of TMV infection for each treatment was calculated by dividing the number of infected plants (symptomatic plus DAS-ELISA-positive plants without symptoms) by the total number of plants in each replicate.

Experiment 5. The four most effective treatments from experiment 4 were chosen for testing in a format that more closely simulated TMV contamination during propagation. Blue Sky liners were transplanted into 3-in. pots containing Metro-Mix 360 5 to 7 weeks prior to treatment. Experiment 5 was a randomized complete block design with 10 plants per treatment and each treatment replicated five times using plants grown from Julian week 19 liners (trial E). Experiment 5 was repeated using plants grown from week 17 liners (trial F). Each razor blade was contaminated by making a single cut on the stem

of TMV-pet7-infected Blue Sky stock plants, soaked in disinfectant solution or water for 1 min, and used to make a transverse cut on the main stem of a single uninfected Blue Sky plant. Separate 100 × 15 mm petri dishes containing 45 ml of one of the following freshly prepared treatments: 0.6% NaOCl (1:10 dilution of household bleach), 20% (wt/vol) NFDm, 20% (wt/vol) NFDm + 0.1% (vol/vol) Tween 20, 1% (wt/vol) Virkon S, or deionized water, were used for each treatment. For the negative control (mock), petunias were cut with new razor blades. Two treatments (10 plants each) were placed at opposite ends of a 7 × 4 carrying tray to maintain a two-cell-wide gap between treatments.

Plants were monitored for symptoms on a regular basis and rated as TMV-pet7-positive when foliar mosaic or color break was first observed. At 61 to 62 dpc, the newest fully expanded flower and associated terminal bud was harvested from a random sampling of 10% of the plants as described for experiment 4 and assayed by DAS-ELISA. If present, symptomatic tissue was sampled. Asymptomatic plants were rated as TMV-pet7-negative, unless the sample tested positive by DAS-ELISA. Percent incidence of TMV-pet7 infection was calculated for each treatment as described above.

Statistical analysis. Data from each trial of experiments 4 and 5 were analyzed independently by analysis of variance (ANOVA) using the general linear model (PROC GLM) of SAS 9.1.3 using square root transformed data (SAS Institute Inc., Cary, NC). Means were separated using Fisher's LSD at $P = 0.05$. Mean percent incidence for each treatment was analyzed to determine if there were any effects due to block, trial, or treatment.

RESULTS

Characterization of petunia tobamovirus isolates collected in Ohio. Symptomatic petunias collected in 2006 were found to be positive for tobamovirus

infection based on DAS-ELISA using commercially available TMV antiserum that is known to cross react with several other tobamovirus species (data not shown). cDNA synthesized from total RNA extracted from six plants was amplified by PCR using tobamovirus-specific primers and directly sequenced. Sequence analysis of the CP ORF of six isolates revealed four different sequences among the six Ohio petunia isolates characterized for this study. Whereas the CP sequences of TMV-pet5, TMV-pet8, and TMV-pet9 were unique among the isolates characterized, the CP sequences of TMV-pet4, TMV-pet6, and TMV-pet7 were identical. BLAST queries with CP sequences of the three unique Ohio isolates and TMV-pet7 revealed that the highest levels of sequence identity were found to two TMV isolates collected in Germany (1853 and 1373-Le1373-208; 27) and wt TMV (Table 1). Multiple sequence alignments of the CP ORFs and deduced amino acid sequences of the Ohio isolates, the most similar TMV isolates identified by BLAST, and sequences of tobamoviruses isolated from petunia were made using ClustalW2 and used to calculate sequence identity (Table 1).

All tobamoviruses collected in Ohio are isolates of TMV as demonstrated by high

levels of CP sequence identity to wt TMV (Table 1). Among the Ohio isolates, TMV-pet7 had the highest levels of CP nucleotide and amino acid sequence similarity to all TMV isolates except TMV-p and Ohio V (Table 1). The CP sequences of TMV-pet7 and TMV-petunia are identical, both having a single amino acid difference (Ser¹⁵⁶) relative to the wt TMV (37; this study). TMV-pet7 was chosen for disinfectant studies in Ohio.

Plant response to tobamovirus inoculation. To establish a tobamovirus–petunia system to screen disinfectants, we tested five tobamovirus species and TMV-pet7 for the ability to infect several vegetatively propagated petunia cultivars. We found differences in virus–cultivar interactions with regard to susceptibility, symptoms, systemic movement, and/or formation of local lesions in inoculated leaves (Table 2). All viruses moved systemically in Baby Red, although only TMV-pet7 caused systemic mosaic symptoms. All viruses moved systemically in Midnight Blue except TSAMV, which produced chlorotic local lesions on inoculated leaves (Table 2). Wt TMV and TMV-pet7 moved systemically in Magenta and White cultivars (wt TMV without symptoms in either cultivar), whereas ToMV and TSAMV induced necrotic local lesions in inoculated

Table 2. Reaction of four petunia cultivars to tobamoviruses used in this study

<i>Tobamovirus</i> spp. or isolate ^y	Surfinia 'Baby Red'	Sanguna 'Midnight Blue'	Surfinia 'Magenta'	Supertunia 'White'
wt TMV	NS/NS ^z	NS/NS	NS/NS	NS/NS
ToMV	NS/NS	NS/NS	NLL/–	NLL/–
PMMoV	NS/NS	NS/NS	NS/–	NS/–
TMGMV	NS/NS	NS/NS	NS/–	–/–
TSAMV	NS/NS	CLL/–	NLL/–	NLL/–
TMV-pet7	NS/M	NS/M	NS/M	NS/NS

^y wt TMV = *Tobacco mosaic virus* strain U1, ToMV = *Tomato mosaic virus*, PMMoV = *Pepper mild mottle virus*, TMGMV = *Tobacco mild green mosaic virus*, TSAMV = *Tropical soda apple mosaic virus*, TMV-pet7 = petunia isolate of TMV.

^z Symptoms on inoculated leaves and upper, uninoculated leaves indicated to left and right of slash, respectively. NS = infected but no symptoms, – = no infection, NLL = necrotic local lesions, CLL = chlorotic local lesions, and M = mosaic. Local and systemic infection confirmed by double-antibody sandwich enzyme-linked immunosorbent assay with washed, inoculated or upper, uninoculated leaves, respectively.

Table 1. Pairwise comparisons of coat protein sequences of select *Tobacco mosaic virus* (TMV) isolates and tobamoviruses isolated from petunia

Virus isolate ^z	Percent nucleotide identity										
	TMV-pet5	TMV-pet7	TMV-pet8	TMV-pet9	wt TMV	1373-Le95-208	1853	TMV-pet	TMV-p	pet-TW	Ohio V
TMV-pet5	---	99.8	99.6	97.9	98.8	99.6	99.2	97.7	95.4	89.0	89.2
TMV-pet7	99.4	---	99.8	98.1	99.0	99.8	99.4	97.9	95.6	89.2	89.4
TMV-pet8	98.7	99.4	---	97.9	98.8	99.6	99.2	97.7	95.8	89.4	89.6
TMV-pet9	98.1	98.7	97.9	---	97.5	97.9	97.5	97.3	95.4	88.5	88.5
wt TMV	98.7	99.4	98.7	98.1	---	98.8	98.3	99.0	96.3	89.4	89.6
1373-Le95-208	98.7	99.4	98.7	98.1	98.7	---	99.2	97.7	95.4	89.0	89.2
1853	98.7	99.4	98.7	98.1	98.7	98.7	---	97.3	95.0	89.0	89.2
TMV-pet	98.7	99.4	98.7	98.1	100	98.7	98.7	---	95.2	88.5	89.0
TMV-p	96.2	96.9	96.9	96.2	97.5	96.2	96.2	97.5	---	89.4	89.0
Pet-TW	94.3	95.0	95.0	94.3	95.6	94.3	94.3	95.6	95.6	---	97.1
Ohio V	94.3	95.0	95.0	94.3	95.6	94.3	94.3	95.6	93.7	96.2	---

^z Virus isolates used in the sequence comparisons listed with the GenBank accession numbers for the nucleotide sequence: TMV-pet5 (GQ370522), TMV-pet7 (GQ370523), TMV-pet8 (GQ370524), TMV-pet9 (GQ370525), wt TMV (NC_001367), 1373-Le95-208 (AJ429082), 1853 (AJ429081), TMV-pet (AB369275), TMV-p (AY029262), pet-TW (EF392659), and Ohio V (AJ429098).

leaves of both cultivars with no subsequent systemic movement. PMMoV and TMGMV infected inoculated leaves of the Magenta cultivar, whereas PMMoV also infected inoculated leaves of the White cultivar. TMV-pet7 induced systemic symptoms in all cultivars except White, in which we found a reduced virus titer based on local lesion assay on *Nicotiana tabacum* cv. Xanthi nc (Table 2 and data not shown). TMV-pet7 also moved systemically and produced leaf mosaic and flower break in Blue Sky and leaf mosaic in White Improved plants (data not shown). Based on these results, we chose TMV-pet7 in Ohio (experiment 1) and wt TMV in Florida for our initial disinfectant screens. Unfortunately, Surfinia 'Baby Red' liners were unavailable at the time of the preliminary trials and the substituted cultivar, Supertunia 'Red', was found not to be susceptible to wt TMV. When Baby Red liners became available, these were used in experiments 2 and 3 with wt TMV in Florida.

Sequential cuts with TMV-contaminated clippers. The purpose of this experiment was to determine the impact of a single TMV contamination event on a series of healthy plants cut after the clippers were contaminated. The order that plants were cut was tracked to determine infection patterns and for how many cuts the inoculum persisted on the clippers. None of the control petunias that were cut prior to contaminating the clippers by cutting a TMV-infected tobacco plant became

infected, indicating that the clippers were initially virus-free. The number of petunia plants that became infected within each set of 20 sequentially cut petunias varied, ranging from 0/20 (C1) to 14/20 (C5), with 23% of plants becoming infected overall (Table 3). The distribution pattern of infected plants within each set of 20 sequentially cut petunia plants also varied (Table 3). In two sets (C4 and C5), the first plant cut (and several subsequent plants) became infected. In the four sets in which at least one plant became infected (C2–C5), multiple plants cut prior to the final infected plant remained uninfected. These results indicate that virus transmission is variable, but also indicate that clippers contaminated by a single cut on a TMV-infected plant can retain sufficient inoculum capable of infecting plants beyond the 38th cut (19th plant).

Initial disinfectant screens. Besides using different cultivars and different tobamoviruses, there were other differences between the three preliminary experiments. In experiment 1, razor blades were contaminated by cutting infected TMV-pet7-infected stock plants, and plants were maintained in liner trays throughout the experiment. In contrast, experiments 2 and 3 used larger plants in 4-in. pots and razor blades that had been dipped into a suspension of purified TMV virions, and several cuts were made on each uninfected plant.

In experiment 1, all 21 Red petunia liners cut with razor blades contaminated with TMV-pet7 prior to a 1-min soak in

water became infected, whereas none of the liners cut with new razor blades (mock) became infected (Table 4). All treatments reduced the incidence of TMV infection (infected/total liners cut per treatment) relative to the water (positive) control (Table 4). A 1-min soak in undiluted household bleach (6% NaOCl) was the only treatment that completely eliminated TMV-pet7 transmission. A 1-min flame treatment from a butane burner (2/21 plants infected) and 1-min contact with a 20% NFDM solution (4/21 plants infected) were the next most effective treatments. Lysol spray to runoff and the following 1-min treatments were less effective at preventing virus transmission: saturated 'TSP', saturated 'TSP-PF', and GreenShield at label rate (1 tsp/qt) (Table 4).

Experiment 2 attempted to use the same active ingredients used in experiment 1, although there were some notable differences due to regional product availability. The NaOCl concentration was 10% in experiment 2, compared to 6% in experiment 1. The ratios of active ingredients in the trisodium phosphate-based disinfectants and sodium carbonate-based, phosphate-free cleaners ('TSP-PF' and 'TSP Substitute') differed between experiments 1 and 2. Furthermore, experiment 2 included four additional disinfectant treatments plus a virus-only control. Based on DAS-ELISA, 13/13 plants from the virus-only treatment and 2/15 plants cut with new razor blades (negative control) became infected (Table 4). All treatments reduced the incidence of TMV infection (Table 4). Saturated 'TSP Substitute' (10/14 plants infected) was less effective than tap water (6/12 plants infected). There was zero incidence of TMV infection with swimming pool chlorine, Lysol, flame, and four disinfectants tested at label rate (GreenShield, ZeroTol, Virkon S, and MENNO Clean).

Four treatments from experiment 2 were replaced by three new treatments in experiment 3, which used the same host system. All virus-only treatment and all water treatment (positive control), but none of the plants cut with new razor blades became infected with TMV (Table 4). All disinfectant treatments reduced the incidence of TMV infection relative to the water control (Table 4). Except for 20% NFDM, all disinfectant treatments repeated in experiment 3 resulted in a higher incidence of TMV infection compared to experiment 2. The least effective treatments in experiment 3 were TwinOxide and ZeroTol (Table 4).

Large-scale disinfectant trial. Experiment 4 used Blue Sky and White Improved liners and inoculum prepared from a TMV-pet7-infected Blue Sky stock plant. Based on its limited efficacy in experiment 1, 'TSP-PF' was eliminated from this experiment. Due to safety issues in a propagation greenhouse, the flame treatment

Table 3. Infection of Surfinia 'Baby Red' petunias following a series of cuts on 20 uninfected plants after contamination of clippers with *Tobacco mosaic virus* (TMV) from a single cut on an infected plant

Plant ^v	Infection from TMV-contaminated clippers				
	C1 ^w	C2	C3	C4	C5
Clean ^x	— ^y	—	—	—	—
1	—	—	—	+ ^z	+
2	—	—	+	+	+
3	—	—	—	+	+
4	—	—	—	+	+
5	—	—	—	—	+
6	—	—	—	—	+
7	—	—	—	—	+
8	—	—	+	—	—
9	—	—	—	—	+
10	—	—	—	—	—
11	—	—	—	—	—
12	—	+	—	+	+
13	—	—	+	—	+
14	—	—	—	—	+
15	—	—	—	—	+
16	—	—	—	—	+
17	—	—	—	—	—
18	—	—	—	—	—
19	—	—	—	—	—
20	—	—	—	—	+

^v Sequence of 20 petunia plants each cut twice with TMV-contaminated clippers.

^w Clipper number contaminated by making a single stem cut on a TMV-infected *Nicotiana tabacum* cv. Xanthi plant prior to making two cuts on each of 20 Baby Red petunia plants.

^x Control petunia plant cut twice with clippers prior to contamination with TMV.

^y Not infected.

^z TMV-infected as measured by double-antibody sandwich enzyme-linked immunosorbent assay of new growth.

was also eliminated. To compromise between the impractical 10-min contact time listed on the Green-Shield label and the 1-min contact time found to be ineffective in experiment 1, we tested both label rate (1 tsp/qt) for 3 min and doubled rate (2 tsp/qt) for 1 min. Although household bleach and swimming pool chlorine completely prevented TMV transmission in preliminary screens, we tested a more commonly used

1:10 bleach solution (0.6% NaOCl) in experiment 4. Based on industry input, we also reduced the trisodium phosphate concentration to the more typically used 3% (wt/vol) and included ZeroTol (1:100 dilution) and 1% (wt/vol) Virkon S.

Flower break symptoms began appearing on Blue Sky plants from the water treatment at 43 dpc (trial A) and 34 dpc (trial C), whereas mosaic symptoms began

appearing on White Improved petunias at 61 dpc (trial B) and 88 dpc (trial D). All asymptomatic petunias, plus randomly selected symptomatic Blue Sky (50 to 51 dpc, trial A; 43 to 46 dpc, trial C) and White Improved plants (49 to 50 dpc, trial B; 36 to 39 dpc, trial D) were assayed by DAS-ELISA. All symptomatic petunias tested were determined to be infected based on DAS-ELISA, but only some of the asymptomatic plants were TMV DAS-ELISA-positive.

To determine if asymptomatic petunias were virus-free, all asymptomatic liners were transplanted into 3-in. pots and maintained in the greenhouse for at least 5 weeks following the initial DAS-ELISA. During this time, all asymptomatic, TMV-infected (DAS-ELISA-positive) plants developed symptoms except one White Improved petunia. Importantly, none of the original DAS-ELISA-negative plants developed symptoms during this time. After this holding period (total of 96 to 102 dpc), 10% of the plants were randomly sampled and retested for TMV infection. All symptomatic plants tested were TMV-positive, and all asymptomatic plants were TMV-negative, as determined by DAS-ELISA.

There were significant effects due to block ($P = 0.05$) and treatment ($P < 0.001$), but there were no significant interactions between trial and treatment. Thus, data were analyzed by trial and were also combined for statistical analysis. The percent incidence of infected petunias resulting from the water treatment (positive control) ranged from 30 to 60% with an overall 46.5% incidence, and none of the mock (new razor blade) treated control plants became infected (Table 5). With the exception of the ZeroTol and 3% 'TSP' treatments in trial C, all treatments significantly reduced the incidence of TMV infection (Table 5). Although none of the

Table 4. Incidence of *Tobacco mosaic virus* (TMV)-infected petunias resulting from preliminary disinfectant screens using virus-contaminated razor blades

Treatment	Incidence of TMV infection		
	Experiment 1 ^v Red	Experiment 2 ^w Baby Red	Experiment 3 ^w Baby Red
No treatment	nt ^x	13/13 ^{y,z}	14/14 ^z
Water	21/21	6/12 ^z	15/15
Bleach (6% NaOCl)	0/21	nt	nt
Swimming pool chlorine (10% NaOCl)	nt	0/14	3/15
Green-Shield (1 tsp/qt)	15/19 ^z	0/15	1/15
Nonfat dry milk (20% wt/vol)	4/21	5/15	0/15
'TSP' (saturated)	14/21	nt	nt
'Tri-Sodium Phosphate (T.S.P.)' (saturated)	nt	1/13	nt
'TSP-PF' (saturated)	18/21	nt	nt
'TSP Substitute' (saturated)	nt	10/14	nt
Flame	2/21	0/15	nt
Lysol (to runoff)	11/21	0/14	nt
ZeroTol (1:100)	nt	0/15	6/15
Virkon S (1% wt/vol)	nt	0/14 ^z	1/15
MENNO Clean (4% vol/vol)	nt	0/15	3/15
TwinOxide (1 ppm)	nt	nt	4/15
TwinOxide (2 ppm)	nt	nt	7/15
Mock (new razor blade)	0/21	2/15	0/15

^v Each razor blade was contaminated by making two transverse cuts on a stem of a TMV-pet7-infected petunia, followed by a 1-min disinfectant treatment as indicated, then used to make a single transverse cut on the stem of one uninfected Supertunia 'Red' petunia liner. Each treatment consisted of 21 liners.

^w Each razor blade was contaminated by soaking in a 1 mg/ml solution of TMV virions for 5 s, followed by a 1-min disinfectant treatment and a 1-min rinse in tap water, then used to make a transverse cut on an average of four stems (3 to 7 stems per plant) of one uninfected Surfinia 'Baby Red' petunia plant. Each treatment consisted of 14 to 15 plants (experiment 2) or 15 plants (experiment 3).

^x Not tested.

^y Number of TMV-infected plants/total plants cut was based upon symptom expression (experiment 1) and double-antibody sandwich enzyme-linked immunosorbent assay (experiments 1 to 3).

^z Final number of plants was less than the number cut due to loss of plants to fungal disease.

Table 5. Incidence of *Tobacco mosaic virus* (TMV) infection of petunia liners cut with TMV-contaminated razor blades treated with a disinfectant prior to cutting each healthy liner

Treatment ^y	Mean percentage incidence of TMV infection				
	Trial A Blue Sky	Trial B White Improved	Trial C Blue Sky	Trial D White Improved	Overall mean
Water	60.0 a ^z	36.0 a	30.0 a	60.0 a	46.5 a
ZeroTol (1:100)	10.0 bc	8.0 b	23.3 ab	10.0 b	12.8 b
'TSP' (3% wt/vol)	16.7 b	4.0 b	13.3 abc	3.3 bc	9.3 b
Green-Shield (2 tsp/qt)	10.0 bc	4.0 b	10.0 bcd	13.3 b	9.3 b
Green-Shield (1 tsp/qt, 3 min)	20.0 b	4.0 b	6.7 cd	3.3 bc	8.5 b
Virkon S (1% wt/vol)	6.7 bc	0.0 b	3.3 d	0.0 c	2.5 c
Bleach (1:10) (0.6% NaOCl)	0.0 c	4.0 b	3.3 d	0.0 c	1.8 c
NFDM (20% wt/vol) + Tween-20 (0.1%)	3.3 bc	0.0 b	0.0 d	3.3 bc	1.7 c
NFDM (20% wt/vol)	0.0 c	4.0 b	0.0 d	0.0 c	1.0 c
Mock (new razor blade)	0.0 c	0.0 b	0.0 d	0.0 c	0.0 c

^y Each razor blade was contaminated for 30 s in sap from a TMV-pet7-infected petunia stock plant, briefly rinsed in deionized water, followed by a 1-min disinfectant treatment (unless noted), then used to make a single transverse cut on the stem of one healthy petunia liner. Each treatment consisted of five replicates of six liners per trial. TMV infection was determined based on symptoms and double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) testing of all nonsymptomatic plants at 55 days post-cutting (dpc) (Sweetunia 'Blue Sky') and 60 dpc (Surprise 'White Improved'). DAS-ELISA negative plants were potted into 3-in. pots. At 96 to 98 dpc (Blue Sky) and 97 to 102 dpc (White Improved), plants were rated for symptoms and 10% were arbitrarily sampled and tested by DAS-ELISA. Mean percent incidence of TMV infection was calculated from the five replicates of each six-plant treatment. NFDM = nonfat dry milk.

^z Data were analyzed for each trial by ANOVA using square root transformed data, but actual percentages are presented. Means within a column followed by the same letter are not significantly different according to LSD ($P = 0.05$).

treatments was completely effective (0% incidence) in all trials, four treatments (20% NFDM, 20% NFDM plus Tween 20, 1% Virkon S, and 0.6% NaOCl [a 1:10 dilution of household bleach]) eliminated TMV infection in two or three trials (Table 5). These four treatments were consistently among the best treatments in both cultivars and all four trials. When the data from all four trials were combined, these four treatments were the most effective at reducing the incidence of TMV infection.

Simulated propagation trial. In experiment 5, the four most effective treatments from experiment 4 (20% NFDM, 20% NFDM plus Tween 20, 1% Virkon S, and 0.6% NaOCl) were compared under conditions that simulated contaminating a cutting tool during the process of taking cuttings from an infected stock plant. Because plants that had been maintained in liner trays for extended periods of time in experiments 1 and 4 became root-bound and required frequent watering, liners were transplanted into 3-in. pots prior to treatment. Plants were maintained in the greenhouse and observed for symptoms consistent with TMV infection. At 61 to 62 dpc, 10% of the plants were randomly sampled and tested by DAS-ELISA. All of the symptomatic plants, but none of the asymptomatic plants tested by DAS-ELISA were TMV-positive.

Percent incidence was calculated as the number of infected plants per treatment based on symptoms and DAS-ELISA results of randomly selected plants. Mean percent incidence for each treatment was analyzed to determine if there were any effects due to block, trial, or treatment. There was a significant effect due to treatment ($P = 0.001$), but no significant effects due to block or trial and no significant interaction between trial and treatment. Data were analyzed by trial and were also combined prior to statistical analysis. The mean percent incidence for the water treatment for trials E and F were 68 and 50%, respectively, for an overall 59% inci-

dence of TMV infection (Table 6). All four treatments significantly reduced the incidence of TMV infection compared to the water (positive) control in both sets of plants, and none of the negative control (new razor blade) cut plants became infected (Table 6). There was no TMV infection from contaminated razor blades treated with 20% NFDM plus Tween 20 or a 1:10 dilution of household bleach (0.6% NaOCl), although these treatments were not significantly different than the 1% Virkon S and 20% NFDM treatments (Table 6).

DISCUSSION

Independent inoculation of several petunia cultivars with different tobamovirus species and TMV isolates resulted in different host responses, including local lesions and symptomless or symptomatic systemic infections. Although this result was not surprising, the failure of several tobamoviruses, including wt TMV, to elicit symptoms in any of the petunia cultivars tested was a significant early finding in this study. The direct impact on our experimental approach was the inability to correlate wt TMV infection with visible symptoms, thus necessitating testing of all plants by DAS-ELISA. For industry, the more profound impact is the demonstration that several (and likely more) petunia cultivars could potentially be symptomless, systemic carriers of TMV or other tobamoviruses. These asymptomatic plants could become an unanticipated source of inoculum, thereby putting other susceptible crops within an operation at risk of infection. Our finding that clippers contaminated by a single cut on a TMV-infected plant could retain infectious virus through cuts on 20 subsequent plants further emphasizes the need for effective disinfectants.

In stark contrast to wt TMV, a petunia isolate of TMV (TMV-pet7) with a CP differing by a single amino acid elicited symptoms on most of the cultivars tested.

Distinct flower color break symptoms elicited by TMV-pet7 on Blue Sky petunias appeared as early as 2 wpc, making this an excellent virus-cultivar combination for testing disinfectants against TMV. Mosaic symptoms on White Improved petunia leaves were considerably slower to develop; thus it was not used in the final experiment.

The preliminary screens were intended to identify the most effective products to test in expanded, replicated trials. Materials tested included those shown to be effective against HLFPV (23) and products that are widely available and/or were suggested by industry cooperators. Due to the unexpected inability of TMV to infect Supertunia 'Red' petunias, and the subsequent delay in obtaining Surfinia 'Baby Red' liners, treatments were repeated with different viruses in different cultivars. There were some notable differences in efficacy of some treatments in the preliminary screens (Table 4). Saturated 'TSP', Green-Shield, and Lysol spray had little effect at reducing the incidence of TMV-pet7 infection (experiment 1), but greatly reduced the incidence of TMV infection (experiments 2 and/or 3). Possible contributing factors could be differences between the viruses, type of inoculum (stem cuts versus purified virus), relative cultivar susceptibility, growing conditions, or a combination of these factors. The source of TMV for the two mock (negative control) plants that became infected in experiment 2 is not known, although contact with adjacent no treatment (virus only) plants is a likely culprit due to close proximity of these two groups of plants. Widespread TMV contamination is not suspected because there were seven treatments that did not contain any infected plants. Although liners were not indexed prior to the experiments, all were produced from virus-indexed stock plant, and none of the mock-treated plants in experiments 1, 3, 4 (two cultivars, four sets of plants), and 5 (two shipments of liners) became infected.

Because the large-scale disinfectant trial (experiment 4) was initiated before preliminary experiments 2 and 3 were completed, treatments were excluded and/or modified based on their efficacy in experiment 1 and industry input. Thus, saturated 'TSP-PF' (sodium sesquicarbonate; 18/21 infected plants) and Lysol (11/21 infected plants) were excluded from experiment 4.

Both products containing trisodium phosphate also contained other ingredients; 'Tri-Sodium Phosphate (T.S.P.)' contained sodium carbonate, and 'TSP' contained sodium sesquicarbonate. The efficacy of saturated solutions of these two products also differed in the preliminary experiments. A saturated solution of 'Tri-Sodium Phosphate (T.S.P.)' (experiment 2) was more effective than a saturated solution of 'TSP' (experiment 1). Differences could be

Table 6. Incidence of *Tobacco mosaic virus* (TMV) infection of petunia plants cut with a TMV-contaminated razor blade treated with disinfectant prior to cutting each healthy plant

Treatment ^a	Mean percentage incidence of TMV infection		
	Trial E	Trial F	Overall mean
Water	68.0 a ^z	50.0 a	59.0 a
Virkon S (1% wt/vol)	6.0 bc	2.0 b	4.0 b
NFDM (20% wt/vol)	4.0 bc	0.0 b	2.0 b
NFDM (20% wt/vol) + Tween 20 (0.1%)	0.0 c	0.0 b	0.0 b
Bleach (1:10) (0.6% NaOCl)	0.0 c	0.0 b	0.0 b
Mock (new razor blade)	0.0 c	0.0 b	0.0 b

^a Each razor blade was contaminated with a single cut on a TMV-infected petunia stock plant prior to treatment, followed by a 1-min disinfectant treatment, prior to cutting the main stem of one healthy Sweetunia 'Blue Sky' plant. Each treatment consisted of five replicates of 10 plants per trial. TMV infection was determined based upon symptoms and randomly testing 10% of the total plants at 61 to 62 days post-cutting by double-antibody sandwich enzyme-linked immunosorbent assay. Mean percent incidence of TMV infection was calculated from the five replicates of each 10-plant treatment. NFDM = nonfat dry milk.

^z Data were analyzed for each trial by ANOVA using square root transformed data, but actual percentages are presented. Means within a column followed by the same letter are not significantly different according to LSD ($P = 0.05$).

due to the second compound or differences between the two systems. Neither was as effective as saturated trisodium phosphate against HLFPV (23). A more commonly used 3% trisodium phosphate solution was tested in experiment 4. Although 3% 'TSP' significantly reduced the incidence of TMV-pet7 compared to the water control, it was not among the most effective treatments. However, based on the manufacturer's label, a 3% (wt/vol) 'TSP' solution contains 2.25 to 2.4% trisodium phosphate and 0.6 to 0.75% sodium sesquicarbonate. Perhaps a solution of pure trisodium phosphate would be more effective, but this raises an issue about availability of materials; the predominant off-the-shelf products in Ohio and Florida contained a second compound. Regardless, our results with solutions containing high and low rates of trisodium phosphate raise a significant concern that this widely used active ingredient may not be as effective a disinfectant for TMV (at least in the most readily available formulations) as has been traditionally thought.

NaOCl was an effective disinfectant over a range of concentrations (0.6 to 10.0%) for preventing tobamovirus transmission from contaminated blades (23; this study). However, its highly corrosive nature and short half-life after dilution (2 h) make it an impractical tool disinfectant for repeated use.

ZeroTol and Virkon S were not tested in experiment 1, but were included in experiment 4. ZeroTol and Virkon S both contain oxidizing agents (hydrogen dioxide and potassium peroxymonosulfate, respectively). Based on experiment 4, Virkon S was significantly more effective at reducing the incidence of TMV infection than ZeroTol with a 1-min contact time. The Virkon S label does not specify how long to treat virus-contaminated tools. O'Neill et al. (30) report complete control on knives contaminated with sap from *Pepino mosaic virus* (PepMV)-infected tomato plants with a 1-min treatment with 1% Virkon S. However, caution is stressed, as results with a potyvirus such as PepMV may not be predictive of results with tobamoviruses.

Although contact with ZeroTol (1:100) for 1 min significantly reduced the percent incidence of virus-infected plants relative to the water control, it was one of the least effective disinfectant treatments in experiment 4. Copes (16) found that higher rates of ZeroTol are required to completely eradicate viable *Botrytis cinerea* spores from metal surfaces. We have not determined whether an increased ZeroTol concentration could also eliminate TMV transmission to petunia with a 1-min contact time.

The Green-Shield label specifies a 10-min contact time to eradicate TMV on contaminated tools, which is not practical for vegetatively propagated annuals, and

thus was not tested. Because there was only a slight reduction in the incidence of TMV-pet7 infection with Green-Shield at label rate (1 tsp/qt) for 1 min in the preliminary screen, we compared an intermediate time versus increased rate in experiment 4. Twice the label rate of Green-Shield at 1 min and label rate for 3 min similarly reduced the incidence of TMV infection, although neither was among the most effective treatments (Table 5). Perhaps a higher concentration of Green-Shield might provide sufficient control as was necessary to completely eradicate *B. cinerea* on metal surfaces (16).

NFDM was found to be one of the most effective treatments for controlling TMV transmission to petunias from virus-contaminated tools, as was shown for HLFPV (23). Although there was not a significant difference between NFDM and NFDM plus a surfactant, the addition of 0.1% Tween 20 reduced the incidence of virus infection to zero from blades contaminated by making a stem cut on an infected plant. Milk has many of the attributes of an ideal disinfectant and can also be used by certified organic and sustainable operations. Milk has been used for years as a disinfectant against TMV in tobacco production (32), and in greenhouses against viruses, although there are few documented trials (22,23,33). In addition, milk causes no runoff or greenhouse re-entry problems, and waste disposal is not an issue. Because of these factors and its excellent performance in these trials, further research into elucidating a mechanism may be warranted.

These studies describe a robust system to test the efficacy of various disinfectant treatments on TMV-contaminated cutting tools. Using these methods, we were able to show that some treatments were significantly more effective than others. The four most effective treatments were consistently among the best treatments in all experiments regardless of cultivar. Although these studies were conducted with tobamoviruses and petunias, the method could easily be adapted to other pathogens and hosts. Based on preliminary screens, products that merit inclusion in future replicated trials include MENNO Clean and TwinOxide. It may also be worth considering testing these and other treatments at above label rates while maintaining the stringent 1-min contact time.

Plant propagators have a zero tolerance policy for virus infection. Thus, even the low incidence of TMV transmission observed with Virkon S and 20% NFDM treatments would be unacceptable. Our studies demonstrate that a single contamination event has the potential to transmit TMV well beyond the first few cuttings. Our results also serve as a potent reminder that petunias infected with TMV or several other tobamoviruses may not always exhibit symptoms.

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