Greenhouse disinfectants – ensuring clean tools for propagation

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Introduction

The wholesale value of floricultural crops in 2008 was estimated at over $4.22 billion; petunias (*Petunia* x *hybrida*) were ranked third among floricultural crops (USDA, 2009). The movement of vegetatively propagated plant material and the continuing introduction of new cultivars and plant species are important routes for introduction of viruses. To minimize losses during vegetative propagation, stock plants are routinely indexed for viruses and major propagators have strict sanitation protocols to minimize the introduction and spread of pathogens.

The most commonly detected virus in petunias in the U.S. is *Tobacco mosaic virus* (TMV) (Nameth, 2002). TMV is transmitted mechanically through contact between infected and uninfected plants, by handling uninfected plants with contaminated hands, or through contaminated tools during propagation. Disinfesting tools is a critical process in preventing spread during propagation.

Only a limited number of products are labeled for use as disinfectants within plant production facilities. An 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).

The TMV-petunia system is ideal because of its economic importance and the difficulty of eradication. There are very few recent studies directly comparing materials for disinfecting hard surfaces contaminated with plant pathogens (Copes, 2004) or tools (Celar et al., 2007; Hayes and Lewandowski, 2009; Kamenova and Adkins, 2004; Lewandowski et al., in press).

Objectives

1. Establish a system for rigorously testing disinfectants for efficacy against important greenhouse pathogens
2. Identify disinfectant treatments that provide effective control of the mechanically transmitted pathogen, *Tobacco mosaic virus* (TMV) on *Petunia x hybrida* (petunia)
3. Screen disinfectants against other pathogen types

Materials and Methods

Petunia sources

Rooted petunia cuttings were received from cooperating growers and were maintained in liner trays or transplanted into 3" or 4" pots and fertilized and watered as needed.

Characterization of petunia isolates of TMV

A number of TMV isolates collected in 2006 from symptomatic petunias in Ohio were characterized. Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen, Inc.). The CP ORF was amplified by RT-PCR using tobamovirus-specific primers (Lewandowski et al., in press; Letschert et al., 2002) and sequenced. The resulting sequences were subjected to BLAST searches and multiple alignments were made to other TMV isolates (**Table 1**). Four vegetative petunia cultivars were inoculated with the TMV petunia isolates and several tobamovirus species that infect solanaceous crops (**Table 2**).

Sequential cuts with a TMV-contaminated clippers

Five sets of 20 petunia plants were cut in sequence (two cuts/plant) with clippers that were contaminated by making a single cut on a TMV-infected tobacco plant. Plants were assayed for infection by TMV (**Table 3**).

Disinfectants

The following products were tested as disinfectants: (i) household bleach (The Clorox Co.), (ii) ZeroTol® (Biosafe Systems LLC), (iii) Green-Shield® (Whitmire Micro-Gen Research Laboratories), (iv) Virkon®S (Antec International Ltd., UK), (v) TSP (The Savogran Co.), (vi) TSP-PF (The Savogran Co.), (vii) Professional Lysol Brand III Disinfectant Spray (Reckitt Benckiser), (viii) nonfat dry milk (NFDM, Nestlé USA), (ix) swimming pool chlorine, (x) MENNO Clean (MENNO-Chemie, Germany), (xi) Tri-Sodium Phosphate (T.S.P.)' (DAP, Inc., Baltimore, MD), (xii) TSP Substitute (Custom Building Products, Seal Beach, CA), and (xiii) TwinOxide® (BTO Solutions, Roseville, CA).

Disinfectant Screening

Preliminary screens utilized 14-21 plants cut with razor blades contaminated by making cuts on a TMV-pet7 infected petunia or dipped in a preparation of TMV virions prior to cutting uninfected petunias (data not shown). Replicated trials were conducted with the more effective materials identified in preliminary screening with or without modifications. Modifications included changes in rate, time and/or the addition of a surfactant (**Table 4**). The four most effective treatments were subjected to screening in a replicated simulated propagation trial (**Table 5**). Plants were determined to be infected based upon DAS-ELISA and/or the development of symptoms. Data was analyzed by SAS 9.1.3 (SAS Institute).

Table 1. Comparison of coat protein sequences of TMV isolates from petunia.

		Percent nucleotide identity										
	Virus Isolate[a]	TMV-pet5	TMV-pet7	TMV-pet8	TMV-pet9	wt TMV	1373-Le95-208	1853	TMV-pet	TMV-p	pet-TW	Ohio V
Percent amino acid identity	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	

[a] Virus isolates with GenBank accession numbers : TMV-pet5-9 (GQ370522-GQ370525), wt TMV (NC_001367), 1373-Le95-208 (AJ429082), 1853 (AJ429081), TMV-pet (AB369275), TMV-p (AY029262), pet-TW (EF392659), Ohio V (AJ429098)

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

	Petunia Cultivar			
***Tobamovirus* sp. or isolate[a]**	**Red**	**Blue**	**Magenta**	**White**
wt TMV	NS/NS[b]	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

[a] 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.
[b] 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. infection confirmed by DAS-ELISA.

Table 3. Infection of petunias following a series of sequential cuts on 20 uninfected plants after a single TMV contamination event.

	Petunia series				
Plant[a]	**1**	**2**	**3**	**4**	**5**
Clean[b]	-[c]	-	-	-	-
1	-	-	-	+	+
2	-	-	+	+	+
3	-	-	-	+	+
4	-	-	-	+	+
5	-	-	-	-	+
6	-	-	-	-	+
7	-	-	-	-	+
8	-	-	+	-	-
9	-	-	-	-	+
10	-	-	-	-	-
11	-	-	-	-	-
12	-	+	-	+	+
13	-	-	+	-	+
14	-	-	-	-	+
15	-	-	-	-	+
16	-	-	-	-	+
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	+

[a] Sequence of 20 petunia plants each cut twice with TMV-contaminated clippers.
[b] Control plant cut twice prior to contamination with TMV
[c] Not infected

Table 4. Replicated disinfectant trials measuring the incidence of TMV infection of petunia liners cut with TMV-contaminated razor blades treated with a disinfectant prior to cutting each healthy liner.

	Mean percentage incidence of TMV-infection				
	Trial A	**Trial B**	**Trial C**	**Trial D**	
Treatment[y]	**Blue Sky**	**White Improved**	**Blue Sky**	**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)	0.0 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 blade)	0.0 c	0.0 b	0.0 d	0.0 c	0.0 c

[y] Each razor blade was contaminated for 30 sec in sap from a TMV-pet7-infected petunia stock plant, followed by 1 min disinfectant treatment (unless noted), a brief rinse in deionized water prior to cutting the stem of a single healthy petunia liner. TSP = Trisodium phosphate, NFDM = nonfat dry milk. Each treatment consisted of six plants replicated 5 times/trial.
[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 ($P = 0.05$).

Table 5. Simulated propagation trial to test the most effective disinfectants.

	Mean percentage incidence of TMV infection		
Treatment[y]	**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)	0.0 c	0.0 b	0.0 b
Mock (new razor blade)	0.0 c	0.0 b	0.0 b

[y] 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 stem of a healthy plant. NFDM = nonfat dry milk. Each treatment consisted of ten plants/treatment and was replicated five times per trial (50 plants).
[z] Data were analyzed for each trial by ANOVA using square root transformed data (actual percentages presented). Means within a column followed by the same letter are not significantly different according to LSD ($P = 0.05$).

Accomplishments:

1. Petunia isolates of TMV that have CP ORFs nearly identical to wt TMV were the only viruses to elicit symptoms on petunia.
2. A two-tiered system consisting of a preliminary disinfectant screen followed by replicated trials to test disinfectants against TMV, one of the most important viral greenhouse pathogens, was established in two geographically disparate locations (Florida and Ohio).
3. 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) 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.
4. Other treatments identified in preliminary screens are candidates for ongoing screening that simulates contamination during the process of taking cuttings.

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Acknowledgements

Salaries and research support provided by state and federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center, the Fred C. Gloeckner Foundation, Inc., and the USDA-ARS Floriculture and Nursery Research Initiative. We thank EuroAmerican Propagators, Proven Winners, Dümmen USA, and Timbuk II Farms for supplying plant material, Syngenta, Ball Horticultural Company, and BTO Solutions for supplying disinfectant materials, EuroAmerican Propagators for providing used, grower-standard clippers, and Agdia, Inc. for supplying TMV ELISA testing reagents. We also thank Landon H. Rhodes and Alissa Kriss for assistance with statistical analysis, Carrie Vanderspool, Nicole Miller, Jeff Smith, Reid Lewis and Lynn Faulkner for excellent technical assistance, and Mike Tiffany, Margery Daughtrey, Darryl Thomas, Mike Klopmeyer, Petra de Rooij, Ann Chase, John Bambara, Bob Stames, Fred Ceballos, Dennis Crum, Deke Jackson, John Gaydos and P. Allen Hammer for advice.