GREENHOUSE SANITATION: EFFICACY OF DISINFECTANTS ON CUTTING BLADES USING *TOBACCO MOSAIC VIRUS* ON *PETUNIA* AS A MODEL

By

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A thesis submitted in partial fulfillment of the requirements for graduation *with research distinction* in the undergraduate College of Food, Agriculture, and Environmental Sciences of The Ohio State University

The Ohio State University

2008

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ABSTRACT

GREENHOUSE SANITATION: EFFICACY OF DISINFECTANTS ON CUTTING BLADES USING *TOBACCO MOSAIC VIRUS* ON *PETUNIA* AS A MODEL

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Petunias (*Petunia x hybrida*) are one of the most popular ornamental crops in the United States. In the early 1990's, the introduction of asexually propagated petunias into the landscape market was associated with increased reports of virus infections, including *Tobacco mosaic virus* (TMV), which is transmitted mechanically. In greenhouses, TMV can be transferred to cutting tools, subsequently infecting healthy stock plants and cuttings. An outbreak of TMV in a greenhouse can quickly spread and devastate entire crops, rendering them unsalable. During vegetative propagation, multiple cuttings are taken from one mother stock plant and multiple stock plants are commonly used. Tool disinfection is critical in preventing the spread of pathogens during this process. At this time, there is no disinfectant that is labeled for greenhouse use against plant viruses with a reasonable contact time.

Commercially available disinfectants and other materials were tested at varying concentrations and contact times to determine the most effective treatments to reproducibly prevent transmission of TMV to healthy plants. Two cultivars of petunia plantlets were tested with eight treatments by dipping razor blades in TMV-contaminated plant sap, then the disinfectant, then making a cut on a healthy plant. Post-inoculation, the petunias were sampled and tested for TMV by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Based on these results, the four most effective treatments were used in a trial to more closely simulate vegetative propagation.

All treatments tested reduced incidence of TMV infection from contaminated razor blades. The most effective one-minute disinfectant treatments in these studies were: 20% non-fat dry milk, 20% non-fat dry milk plus surfactant, 1:10 household bleach, and 1% Virkon®S.

Acknowledgments

A word of appreciation is extended to my research advisor Dr. Dennis Lewandowski, my professor and friend Dr. Landon Rhodes, and my academic advisor Dr. Mike Boehm.

This research was funded in part by an Ohio Agricultural Research and Development Center (OARDC) SEEDS grant, an OSU Honors and Scholars Research Grant, and the USDA Floriculture and Nursery Research Initiative. Gratitude is also expressed to EuroAmerican Propagators for plant material, and to Dümmen USA and Timbuk II Farms for providing quality petunias for the bulk of these experiments.

Thanks also to Debra Bermann and the Brazil Research Exchange program; Alyssa Kriss (the Statistician); Mike Kelly (the Grinder); David Snodgrass (the Sprayer); Carola de la Torre Cuba, Jessica Schafer, and Chris Woltjen for support, help, advice, and friendship; Jim Gibson and Stacy Decker at Timbuk Farms; Dr. P. Allen Hammer at Dümmen USA/Red Fox; and Bart Hayes at OSU Horticulture and Crop Science and at home.

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INTRODUCTION

The floriculture industry was valued at $5.07 billion in wholesale sales in 2003, 55% of which was attributed to annual bedding plants [Daughtrey et al, 2005]. Petunias (*Petunia x hybrida)* are the third most profitable ornamental crop in the United States [Nameth, 2002]; 921,000 square feet of greenhouse production space is utilized for the crop in Ohio, ranking the state fourth nationally in petunia production. In 2007, United States Department of Agriculture (USDA) rankings of floriculture crops listed wholesale petunia sales of $111,677,000 in the United States, behind poinsettias and orchids [Tambascio, 2008]. In a recent survey, 43% of responding greenhouse growers listed *Petunia* as the top genera grown by numbers of plants sold [Tambascio, 2007].

Asexual or vegetative propagation techniques ensure that the propagated plantlets, often called liners, are identical to the mother plant, as they share the same genetic makeup [Ingram, 1993]. In the early 1990's, the introduction of vegetatively propagated petunias into the landscape market was associated with increased reports of virus infections. New hybrid cultivars, such as the original *Petunia* hybrid 'Surfinia' from Japan, require vegetative propagation [Chung et al, 2007].

Of the over 130 viruses known to infect petunias, *Tobacco mosaic virus* (TMV) is the most commonly detected virus in the United States [Nameth, 2002]. TMV, a member of the genus *Tobamovirus,* is a rigid rod-shaped virus approximately 18 nm in diameter and 300 nm in length [Lewandowski, 2005]. TMV has a very wide host range and is estimated to infect over 350 species of plants [Moorman, 2007]. Viral transmission occurs by mechanical means; by contact between plants or by workers physically moving the virus from plant to plant by touching infected plant material [Lewandowski, 2005 and Phillipson et al, 2005]. Once inside the plant, the virus' protein coat is removed and the nucleic acid directs the plant cell to produce more viral RNA. TMV virions are

exceptionally stable and are able to survive for months outside the host, such as on greenhouse benches, tools, and surfaces [Moorman, 2007]. Transmission has also been shown to occur during watering, where the watering can brushed against the plants, and during experimental sampling, if gloves are not changed regularly [Phillipson et al, 2005]. Because of its economic importance and the difficulty of eradicating TMV, it is an ideal model system to study greenhouse sanitation.

Symptoms of TMV on petunia include mosaic patterns on leaf tissue, flower color break, stunting, and leaf rugosity (Figure 1). Vegetatively propagated petunias are vigorous, and require high levels of fertilization and watering with adequate pH; if these conditions are not met, plants may exhibit symptoms of these abiotic problems that resemble TMV symptoms [Lesemann, 1996]. TMV causes millions of dollars in losses in the floriculture industry annually. In Georgia, virus diseases were responsible for an estimated $1.89 million in ornamental crop losses for 2006 [Williams-Woodward, 2000]. Because of the intensity of production of ornamentals and the high plant density, a virus outbreak in a greenhouse can quickly spread and devastate entire crops, rendering them unsalable [Albajes et al, 1999].

The mechanical nature of viral transmission leads to the risk of spread by cutting tools. In greenhouse situations with TMV-infected stock plants used for cuttings, it can be transferred easily from the stock plant to the cutting tool, and thus between infected and healthy stock plants. During vegetative propagation, multiple cuttings are taken from one mother stock plant and multiple stock plants are commonly used. Disinfesting tools is a critical process in preventing the spread of disease during this process. At this time, there is no disinfectant that is labeled for greenhouse and tool use against viruses with a reasonably short contact time; most are labeled for ten-minute tool soaks.

University extension specialists list various chemicals for disinfection of cutting tools against viruses of ornamental plants, such as 10% bleach solution [Nameth, 2002] or Virkon®S [Pundt, 2003],

but for varying lengths of time; and recommend various chemicals for hand sanitization, such as tri-sodium phosphate (TSP) or milk [Albajes et al, 1999 and Pundt, 2003].

Nester et al. [1995] list the ideal germicidal chemical selection factors as level of toxicity to humans, activity in presence of organic matter, compatibility with the material being treated, presence and toxicity of residues, cost and availability, storage and stability, and environmental risk. If used when plants are present, greenhouse disinfectants should not cause phytotoxicity. The disinfectant should also be broad spectrum, and be effective against a wide range of plant pathogens. This research focused on controlling an important virus disease; however, efficacy against bacterial and fungal pathogens should also be considered. The ideal disinfectant would reproducibly prevent the spread of TMV from tools to healthy plants with a short contact time—ideally no more than one minute. This would enable greenhouse growers to soak one tool while another is being used, minimizing downtime spent waiting for tool sanitation. There is a lack of comprehensive, independent, replicated studies to determine which of the commercially available disinfectants or other treatments are most effective to minimize the spread of plant viruses, and what methods should be used to get the best results from those disinfectants.

The most widely used greenhouse disinfectants include those produced from quaternary ammonium chloride salts, hydrogen dioxides, chlorine bleach, and 70% alcohol [Smith, 2007]. Alcohol, although effective and fast-acting, is not practical in a greenhouse situation due to its flammability.

Quaternary ammonium salts are non-toxic enough that they are often used on food preparation surfaces. They are inactivated by anionic soap or detergents, and are a positively charged (cationic) detergent. These chemicals reduce the surface tension of liquids and help wash away dirt, enabling microorganisms to be physically removed. Because of their positive charge, they are attracted to cell surfaces, and are known to destroy bacteria and enveloped viruses by coagulation and

denaturation of proteins [Nester et al, 1995]. In addition, the compound's activity decreases when mixed with hard water or organic material [Jeffrey, 1995].

The peroxygen compounds are readily biodegradable and less toxic than traditional alternatives. However, their spectrum of activity is limited; the common enzyme catalase degrades the compound into water and oxygen. The presence of this oxygen inhibits the growth of anaerobic organisms [Nester et al, 1995]. Peroxygen compounds are considered, at certain concentrations, to be bactericidal and virucidal, though non-enveloped viruses may be resistant [Dvorak, 2005]. Experiments have shown Virkon®S, the chemical used in this experiment, to be effective to some degree against bacterial and fungal plant pathogens [Howard et al, 2007]. In addition, the compound has a short half-life and degrades quickly once diluted with water.

Disinfectants produced from halogens are inexpensive and readily available. Chlorine destroys all types of microorganisms and viruses, but is irritating to the skin and corrosive to tools. Organic matter and impurities can neutralize chlorine's activity by consuming free ions, necessitating a rinse prior to use if excessive plant material is present on the cutting tool. In addition, working solutions have a short half-life (two hours) and are light sensitive [17]. Chlorine compounds are electronegative; they function through denaturing pathogen proteins [Nester et al, 1995]. Sodium hypochlorite has been shown to negate fungal spores' viability on greenhouse surfaces, such as plastic, wood, and metal [Copes et al, 1996].

Milk has been shown to control some powdery mildew fungi, and is especially popular in organic agriculture [Bettiol, 1999], and skimmed milk contains proteins which encapsulate plant virions, preventing transmission [Albajes et al, 1999]. In addition, industry professionals often recommend the use of a solution of non-fat dry milk (NFDM) after hand washing to prevent plant virus transmission, or as a spray for transplants to prevent transmission from hand to plant [Henn, 2004 and Seebold, 2008]. Tri-sodium phosphate (TSP) has been used for years, usually as a hand-

washing agent for workers to prevent the spread of plant viruses. In a study where TSP was mixed with sap from TMV-infected plants and then rubbed on an indicator plant, 5%, 10%, and 20% TSP inactivated the virions after five minutes [Brock, 1952]. These results, combined with the prevalence of tri-sodium phosphate use within the floriculture industry, necessitated its inclusion in this study; an industry recommended 3% TSP solution (various manufacturers) was utilized for these studies.

In Florida, tool sterilization has been tested against *Hibiscus latent Fort Pierce virus* (HLFPV), a Tobamovirus infecting hibiscus. In this study, Kamenova et al. [2004] found that a one-minute contact time with 10% Sodium hypochlorite (swimming pool chlorine) or 20% NFDM prevented virus transmission to healthy plants.

In a preliminary study conducted summer, 2007, small numbers of petunias (19-21 in Study I; 8 in Study II) were utilized in two separate tests. From these trials, it was determined that ZeroTol®, bleach, and NFDM were the most effective disinfectants tested, and saturated TSP was one of the least effective (Figures 2-6). GreenShield®, a popular industry quaternary ammonium salt-based product was not effective at a one-minute soaking time. This necessitated changes in concentration or contact time in an attempt to improve efficacy of GreenShield® and TSP. In addition, a surfactant was mixed with NFDM in an attempt to provide better contact between the tools and the disinfectant.

OBJECTIVE, PHASE I

Screening of Disinfectant Treatments against TMV

During this phase of experimentation, the objective was to identify the most effective treatments for reducing transmission of TMV from contaminated cutting tool to healthy plants. The disinfectants were tested at one minute (except GreenShield® at label concentration, which, due to its limited efficacy in preliminary screenings, was tested at three minutes), and the razor blades were rinsed prior to disinfection.

MATERIALS AND METHODS, PHASE I

Plant Material and Crop Records

Unrooted cuttings of two susceptible petunia cultivars were utilized: *Petunia x hybrida* 'Sweetunia® Blue Sky' (Cultivar A) and *P. x hybrida* 'Surprise® White Improved' (Cultivar B) were donated by Dümmen USA and rooted at Timbuk II Farms in Granville, Ohio. The six-week-old liners were delivered to The Ohio State University on a biweekly schedule. Groups of six plants (biological replicate) were arranged in 102-cell liner trays (17 x 6) [figures 7 and 8]. Trays were labeled with block number, cultivar, and start date with permanent marker on tray edge. Biological replicates were then randomly selected and subjected to one of the ten treatments including positive (water) and negative (no virus) controls. Each treatment was replicated five times per trial, with two trials per cultivar. The first plant in each biological replicate was tagged with treatment number, block number, and cultivar.

Plants were placed in the greenhouse on an isolated bench (Figure 9) and fertilized periodically with 300 parts per million (ppm) 20-10-20 fertilizers (Peters Professional® General Purpose™, The Scotts Company, USA). Plants were watered from overhead using either hand watering or an

automatic sprinkler system as needed. Soil temperatures in the greenhouse ranged from 17.1° C to 30.2° C and air temperatures ranged from 17.9° C to 33.5° C; relative humidity ranged from 56.8% to 82.0%. Blue sticky cards for insect population monitoring and trapping were placed among the canopy and checked weekly; insecticides were sprayed as needed to maintain low insect populations in the greenhouse.

Inoculum and Blade Contamination

Symptomatic leaf tissue from TMV-infected petunias was tested for TMV infection with an ImmunoStrip® (Agdia, Inc., Elkhart, Indiana, USA) immediately prior to use and 5 g tissue was ground with a mortar and pestle in 35 mL de-ionized water for inoculum. Thirty (30) mL of inoculum was transferred to a 100 x 15 mm plastic, disposable Petri dish. Six single-edged surgical carbon-steel razor blades (1.5L x 0.75W in., 0.12 in. thick, ASR Co., USA) were bound together with a 2" binder clip (Figure 10). Chemical treatments (Table 1) were mixed (Table 2) with de-ionized water immediately prior to initiating each trial. 20 mL of each mixture was dispensed into five 60 x 15 mm plastic, disposable Petri dishes. The bound groups of blades were dipped into the inoculum for 30 seconds by standing the blades on end in the Petri dish (Figure 11). The group was then blotted on a clean paper towel by gently touching the corner of the group opposite where the cut was to be made; this removed excess sap prior to rinsing. The group was then briefly rinsed in 25 mL clean de-ionized water in a 60 x 15 mm Petri dish to remove excess plant material. The blade groups were soaked in the chemical treatment for one minute (with the exception of GreenShield® at label concentration, which was soaked for three minutes). The blades were unclipped onto a clean paper towel, and each used to make a single cut on a single petunia liner. The positive control consisted of plants cut with blades dipped in infected sap, rinsed, and then soaked in water for one minute. Negative controls consisted of plants cut with clean, uncontaminated razor blades.

Plant rating and data analysis

The first symptoms appeared as flower color break on cultivar A at 34 days post-treatment, and appeared on additional new plants through day 98 post-treatment. On cultivar B, a white cultivar, symptoms appeared as leaf mosaic on new, expanding leaves at day 61 post-treatment and appeared on additional plants until day 102 post-treatment. Plants of different biological replicates were prevented from touching one another for the course of the experiment (Figures 7, 9).

At 55 days post-treatment (cultivar A) and 60 days post-treatment (cultivar B), the newest fully expanded flower and associated terminal bud of each plant were sampled by cutting with a sterile blade. If present, symptomatic tissue was sampled. Tissue from each asymptomatic plant as well as one symptomatic plant per block was sampled. Symptomatic plants that were not sampled were rated as infected. Tissue was ground with 3 mL General Extraction Buffer (Agdia, Inc.) for DAS-ELISA according to supplier's protocols.

All asymptomatic plants, (DAS-ELISA negative and positive), were potted into 3" square pots at day 62 (Cultivar A and B week 13 tested with DAS-ELISA) and day 69-71 (Cultivar A and B week 15 tested with DAS-ELISA) post-treatment. DAS-ELISA positive, asymptomatic plants were placed together in a pot shuttle apart from DAS-ELISA negative, asymptomatic plants. The DAS-ELISA positive group was monitored every 2-3 days for symptoms. Once symptoms had appeared on all but one plant (a Cultivar B), the plants were rated again for symptoms and arbitrarily sampled and tested by DAS-ELISA (Table 3).

Percent incidence was calculated for each biological replicate from the number of symptomatic plus ELISA positive asymptomatic plants divided by the total number of plants and multiplied by 100.

Data were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC). Analysis of variance was conducted to determine statistical significance of block, cultivar, treatment, and date using square root transformed data. Means were separated using Fisher's LSD at P=0.05.

RESULTS, PHASE I

Experiments were conducted with two cultivars and on two different dates to determine if there were any effects that might be due to cultivar and/or environmental conditions that differed throughout the season. Phase I data (week 13 and 15 plants) were analyzed to determine if there were any significant effects due to block, cultivar, date and treatment, and whether there were interactions between cultivar, date, and treatment. There were significant effects due to block (P=0.01), cultivar (P=0.05) and treatment (P<0.001) (Table 4). However, there were no significant interactions between cultivar, date and treatment (Table 4).

Other than ZeroTol and TSP in week 15, cultivar A, all treatments significantly reduced the incidence of TMV infection at both dates and cultivars (Table 5). When experiments were combined, all treatments were found to have significantly reduced the incidence of TMV infection. The most effective treatments were NFDM, NFDM with Tween-20®, Virkon®S, and bleach, which were significantly different than the other four treatments (Table 5). Thus, NFDM, NFDM with Tween-20®, Virkon®S, and bleach were chosen for Phase II.

OBJECTIVE, PHASE II

Simulated Propagation

The goal of this phase of experimentation is to closely simulate the vegetative propagation process which is commonly used to commercially produce petunias for the floriculture industry. Each razor blade was contaminated by making a single cut on a TMV-infected petunia stem, simulating the process of taking a cutting for propagation. In contrast to Phase I tests, the rinse step was eliminated to more closely replicate grower practices. Each blade was then placed into the disinfectant treatment for one minute.

Plants used for Phase II were older than in Phase I, and had been potted in 3" pots and heavily fertilized to maintain vegetative, healthy growth.

MATERIALS AND METHODS, PHASE II

Plant Material and Crop Records

Sixteen-week-old petunias in 3" pots (potted at nine weeks) of cultivar A ('Sweetunia® Blue Sky') were utilized. Groups of ten plants (biological replicate) were tested for each of the four most effective disinfectant treatments from Phase I (Table 5), plus positive (water) and negative (no virus) controls. Each treatment was replicated five times per trial, with two trials performed on liners received Julian week 17 and Julian week 19.

Plants were maintained in the greenhouse and fertilized periodically with 300 parts per million (ppm) 20-10-20 fertilizers (Peters Professional® General Purpose™, The Scotts Company, USA); overhead irrigation was used. Soil temperatures in the greenhouse ranged from 17.1°C to 33.3°C and air temperatures ranged from 19.3°C to 36.7°C; relative humidity ranged from 60.2% to 82.0%. Insecticides were sprayed as needed to maintain low insect populations in the greenhouse.

Inoculum and Blade Contamination

Twelve symptomatic, TMV-infected petunia stock plants (cultivar A) were utilized for inoculum for this phase. Eight of these twelve had previously tested positive for TMV infection with DAS-ELISA, OD_{405} >3.367; the other four plants were tested for TMV infection with a TMV ImmunoStrip® (Agdia, Inc.) immediately prior to use (Table 6). Flower shoots with terminal symptomatic flowers were arbitrarily removed from the plants (1-2 per plant); with an average shoot length of 8". Each shoot was used to contaminate single-edged razor blades by cutting through the stem tissue. Each blade was used to make a single cut on one stem of a shoot, then soaked in the disinfectant treatment for one minute and finally used to make a single cut on the main stem of a potted petunia. All chemical dilutions were prepared fresh with de-ionized water immediately before use. Positive controls consisted of plants cut with blades contaminated as above and then soaked in water for one minute. Negative controls consisted of plants cut with clean, uncontaminated razor blades.

Plant Rating and Data Analysis

Symptoms began appearing as flower color break as early as 12 days post-treatment, and continued appearing through day 62 post-treatment. Symptomatic plants were rated as TMV-positive, and asymptomatic plants were rated as TMV-negative. At 61-62 days post trial, the newest fully expanded flower and associated terminal bud was harvested from a random sampling of plants by cutting with a sterile blade (one out of every 10 plants). If present, symptomatic tissue was sampled. Tissue was ground with 3 mL General Extraction Buffer (Agdia, Inc.) for DAS-ELISA.

Percent incidence was calculated for each biological replicate from the number of symptomatic plants divided by the total number of plants and multiplied by 100. All symptomatic, tested plants were confirmed to be infected with TMV based on DAS-ELISA. All asymptomatic plants were found to be

TMV-negative based on DAS-ELISA. Data were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC). Analysis of variance was conducted to determine statistical significance of block, treatment, and date. Means were separated using Fisher's LSD at P=0.05.

RESULTS, PHASE II

Phase II TMV-incidence data (week 17 and 19 plants) were analyzed to determine if there were any significant effects due to block, date, and treatment, and whether there were interactions between date and treatment. There was a significant effect of treatment ($P<0.001$); however, there was no significant effect of date or interaction between date and treatment (Table 7). Data were analyzed by date and combined (Table 7).

All four treatments significantly reduced the incidence of TMV infection (Table 8). There was zero incidence of TMV infection for two treatments (NFDM with Tween-20® and bleach).

DISCUSSION

The two *Petunia x hybrida* cultivars used for these experiments ('Sweetunia® Blue Sky' and 'Surprise® White Improved') showed different symptoms as a result of TMV infection. 'Sweetunia® Blue Sky' displayed a characteristic and easily recognizable flower color break as early as day 36 of the Phase I experiments, and as early as day 26 of the Phase II experiments. 'Surprise® White Improved' displayed a primary symptom of leaf mosaic, which began day 88 for Phase I. Because of the long delay in symptoms on 'Surprise® White Improved', it was not used for Phase II experiments. The reliability and ease of recognition of the flower color break symptom on 'Sweetunia® Blue Sky' make it an ideal model for TMV.

Because the plants remained in liner trays for an extended period in Phase I, they were difficult to maintain. Their root systems quickly became pot-bound, and they required frequent watering to prevent the root mass from drying out. In addition, their nutrient needs could not easily be met, and they were susceptible to *Botrytis cinerea* infections. Daily, crop residue and dying plant material had to be carefully removed with sterile forceps to prevent botrytis stem rot. In light of these difficulties, potted plants were used for Phase II experiments. However, these provided challenges, as well. The plants quickly overgrew their space, and more space was needed to prevent the plants from touching.

The addition of a surfactant (0.1% Tween-20®) to NFDM had no significant effect on the percentage of infected plants. Milk is safe for workers, plants, equipment, and beneficial insects, and can be used by certified organic and sustainable operations. In addition, it 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. NFDM, both with and without Tween-20®, was utilized in Phase II experiments.

Bleach, although effective in this trial, caused severe corrosion of the razor blades. After the one-minute soak in a 1:10 bleach solution (0.6% NaOCl), the blades were speckled with rust. In a greenhouse setting where expensive pruning shears or blades are used repeatedly for many seasons, this corrosion may prove undesirable for growers. Also, when diluted, bleach has a short half-life which may prove impractical for growers, as they would need to replace the solution periodically throughout the day. Despite the detrimental effects (corrosion) on the blades, bleach was one of the most effective treatments to reduce TMV transmission, and was thus included in Phase II experiments.

ZeroTol® and Virkon®S are both oxidizing agents. However, Virkon®S was significantly more effective at reducing incidence of TMV infection than ZeroTol®, possibly due to the instability of ZeroTol® in the presence of organic matter. In this experiment, ZeroTol® was mixed prior to use; an estimated one hour lapsed between mixing and completion of use.
Because Virkon®S significantly reduced TMV transmission, it was subsequently included in Phase II experiments.

The incidence of TMV infection in plants cut with TMV-contaminated blades treated with GreenShield® at label rate were not significantly different at three minutes than blades treated with GreenShield® at double the label rate for one minute. The GreenShield® label recommends a ten-minute soak for tools, which was not tested. Neither GreenShield® treatment was tested in Phase II.

In preliminary tests, saturated TSP (~20%) was not as effective at reducing TMV transmission in *Petunia* as it was for a Tobamovirus in a woody host [Kamenova et al, 2004]. The TSP concentration was reduced to 3% per discussions with industry representatives. 3% TSP significantly reduced the incidence of TMV infection, but was not among the most effective treatments. Therefore, TSP was not included in Phase II.

These studies demonstrate a robust system to test various disinfectant treatments for treating virus-contaminated cutting tools. Using these methods, we were able to show that some treatments were significantly more effective than others. Although these studies were conducted with one host-virus combination, the method could easily be adapted to other pathogens and hosts. The four treatments tested in Phase II were not statistically significant from one another. However, greenhouse growers have a zero tolerance policy for virus transmission during propagation, so even the two and four percent transmission rates for Virkon®S and 20% NFDM would be unacceptable for growers. Of the two treatments which provided zero percent incidence of transmission, bleach is corrosive to cutting tools, and thus non-fat dry milk is the preferred treatment. The method by which milk is able to prevent transmission is unknown; this warrants further study. The effect of different surfactants or surfactant concentrations also merits further exploration.

Table 1. Treatments Used for Disinfection.

Treatment	Manufacturer	Chemical Class	Contact Time(min)	Concentration
*De-ionized Water	-	-	1	-
*Bleach	The Clorox Company, USA	Halogen	1	0.6%
Tri-sodium phosphate (TSP)	Various	phosphate	1	3% wt/vol
GreenShield®	Whitmire Microgen Research Laboratories, Inc., USA	Quaternary ammonium salt	3	1 tsp/1 qt
GreenShield®	Whitmire Microgen Research Laboratories, Inc., USA	Quaternary ammonium salt	1	2 tsp/1 qt
ZeroTol®	Biosafe Systems, LLC, USA	Hydrogen dioxide	1	1:100
*Non-fat dry milk (NFDM)	Nestlé USA, Ohio, USA	Milk product	1	20% wt/vol
*NFDM + Tween-20®	Nestlé USA, Ohio, USA + Cayman Chemical Co., USA	Milk product +polysorbate 20	1	20% wt/vol (milk) + 0.1 % Tween-20®
*VirkonS®	Antec International Ltd., UK	Sodium chloride	1	1%

* Chemicals used for both Phase I and II are marked with an asterisk (*). Those without were used only for Phase I.

Table 2. Chemical dilutions for Phase I and II experiments.

Treatment Number	Treatment	Volume	Concentration	Concentrate	De-ionized water
1*	Water	125 mL	-	-	125 mL
2*	Bleach	125 mL	0.6%	12.5 mL	112.5 mL
3	TSP	125 mL	3% wt/vol	3.75 g	125 mL
4	GS	125 mL	1 tsp/qt	0.651 mL	124.35 mL
5	GS x 2	125 mL	2 tsp/qt	1.302 mL	123.7 mL
6	ZeroTol®	125 mL	1:100	1.25 mL	123.75 mL
7*	NFDM	125 mL	20% wt/vol	25 g	125 mL
8*	NFDM + T20	125 mL	20% wt/vol + 0.1% T20	25 g NFDM + 125 μL T20	125 mL
9 *	Virkon®S	125 mL	1%	1.25 g	125 mL

TSP = Tri-sodium phosphate
GS = GreenShield®
GS x 2 = GreenShield® at twice label rate
NFDM = non-fat dry milk
T20 = Tween-20®
*Disinfectants used for both Phase I and II are marked with an asterisk (*). Those without were used only for Phase I.

Table 3. Julian dates for Phase I and II Experiments.

Phase I Julian week plants received	CV*	Trial start	1st symptoms	Last plant that developed symptoms	DAS-ELISA sampling	Potted	DAS-ELISA sampling	Teardown of trial
13	A	86	129	178	141-142	148	182	192
13	B	87	148	182	149-150	156	189	189
15	A	100	134	198	154-155	169-171	198-200	198-200
15	B	101	189	213	161-162	175-6	189-200	198-200
Phase II Julian week plants received		Received	Potted	Trial start	1st Symptoms	Last plant that developed symptoms	DAS-ELISA sampling	Teardown of trial
17		115	136-138	184	198	246	246	246
19		128	156	191	213	248	248	248

*CV (cultivar) A: *Petunia x hybrida* 'Sweetunia® Blue Sky'. CV B: *Petunia x hybrida* 'Surprise® White Improved'.

Table 4. Phase I overall analysis of variance; incidence of TMV infection in petunia liners cut with razor blades contaminated with sap from TMV-infected petunias and treated with disinfectant prior to cutting each healthy liner.[†]

	Incidence	
Source	**DF**	**Pr > F**
BLOCK	4	0.01
DATE	1	NS[‡]
CV	1	0.05
TMT	9	0.001
CV*DATE	1	NS
DATE*TMT	9	NS
CV*TMT	9	NS
CV*DATE*TMT	9	NS

[†] Analysis was made on square root transformed data.

[‡] NS, not significant at $P = 0.05$

Table 5. Phase I incidence of TMV infection of petunia liners cut with TMV-contaminated razor blades treated with a disinfectant prior to cutting each healthy liner.[†]

	WEEK[‡] 13		WEEK 15		
TREATMENT	**CV[§] A**	**CV B**	**CV A**	**CV B**	**Combined**
WATER	60.0 a[¶]	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%)	16.7 b	4.0 b	13.3 abc	3.3 bc	9.3 b
GreenShield (2X)	10.0 bc	4.0 b	10.0 bcd	13.3 b	9.3 b
GreenShield (3 min)	20.0 b	4.0 b	6.7 cd	3.3 bc	8.5 b
Virkon®S S (1%)	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
Non-fat dry milk (20%) +T20*	3.3 bc	0.0 b	0.0 d	3.3 bc	1.7 c
Non-fat dry milk (20%)	0.0 c	4.0 b	0.0 d	0.0 c	1.0 c
CONTROL	0.0 c	0.0 b	0.0 d	0.0 c	0.0 c

[†] Each razor blade was dipped in sap from a TMV-infected petunia stock plant and used to cut a single healthy liner.

[‡] Julian week that liners were received from supplier.

[§] CV = *Petunia x hybrida* cultivar 'Sweetunia® Blue Sky' (A); 'Surprise® White Improved' (B)

[¶] Means within a column followed by the same letter are not significantly different ($P = 0.05$). Analysis was made on square root transformed data, but actual percentages are presented.

*T20, 0.1% Tween-20.

Table 6. DAS-ELISA Results for plants used for Inoculum, Phase II

Cultivar	Plant Number	Symptomatic	OD_{405}	ImmunoStrip® result	OD_{405} GEB*	OD_{405} HPM**
A	1-1-a	Yes	>3.500	n/a	0.2305	0.234
A	1-9-b	Yes	>3.500	n/a	0.2305	0.234
A	3-6-e	Yes	>3.500	n/a	0.2305	0.234
A	3-5-d	Yes	>3.500	n/a	0.1705	0.155
A	5-6-c	Yes	>3.500	n/a	0.1425	0.122
A	5-3-a	Yes	0.801	positive	0.087	0.090
A	7-3-c	Yes	n/a	positive	-	-
A	9-1-c	Yes	n/a	positive	-	-
A	9-3-d	Yes	n/a	positive	-	-
A	9-1-e	Yes	>3.500	n/a	0.131	0.205
A	3-3-b	Yes	3.367	n/a	0.131	0.205
A	1-3-d	Yes	>3.500	n/a	0.131	0.205

OD_{405}: optical density at 405 nm.
*GEB = General Extraction Buffer (Agdia®, Inc.)
** Healthy plant material

Table 7. Phase II overall analysis of variance; incidence of TMV infection in petunia liners cut with razor blades contaminated by cutting on a stem of a TMV-infected and treated with disinfectant prior to cutting each healthy liner.

		Incidence
Source	**DF**	**Pr > F**
BLOCK	4	NS[†]
DATE	1	NS
TMT	5	0.0001
DATE*TMT	5	NS

[†] NS, not significant at $P = 0.05$

Table 8. Phase II incidence of TMV infection of *Petunia x hybrida* 'Sweetunia® Blue Sky' plants cut with a TMV-contaminated razor blade treated with disinfectant prior to cutting each healthy plant.[†]

	WEEK[‡]		
TREATMENT	**17**	**19**	**COMBINED**
WATER	68.0 a[§]	50.0 a	59.0 a
1% Virkon®S®	6.0 b	2.0 b	4.0 b
20% Non-fat dry milk	4.0 b	0.0 b	2.0 b
20% Non-fat dry milk + T20	0.0 b	0.0 b	0.0 b
Bleach (1:10)	0.0 b	0.0 b	0.0 b
CONTROL	0.0 b	0.0 b	0.0 b

[†] Each razor blade was contaminated with a single cut on a TMV-infected petunia stock plant and used to cut a single healthy liner.
[‡] Julian week that liners were received from supplier.
[§] Means within a column followed by the same letter are not significantly different ($P = 0.05$).

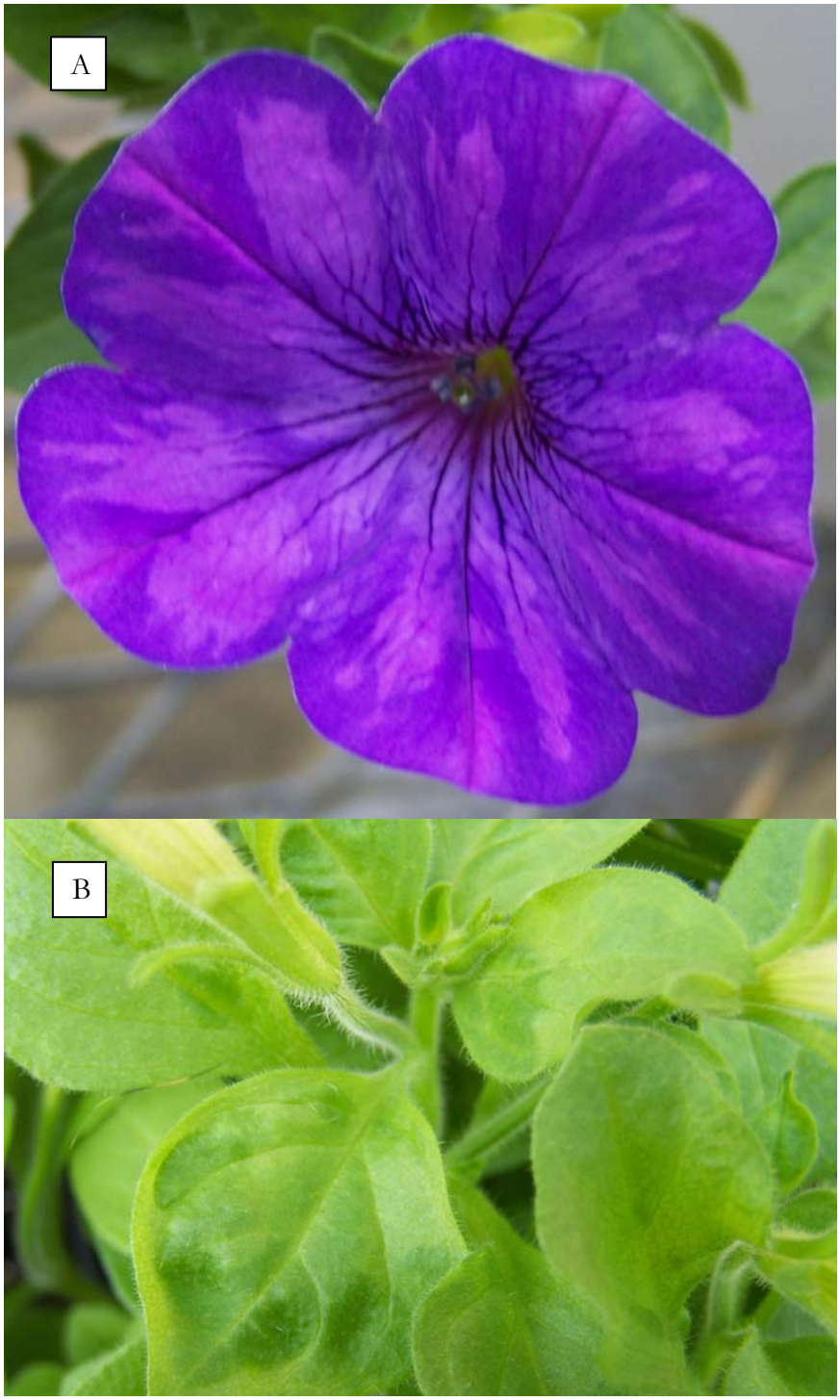

Figure 1. TMV symptoms (A) flower color break, on Cultivar A, *Petunia x hybrida* 'Sweetunia® Blue Sky' and (B) leaf mosaic, on Cultivar B, *Petunia x hybrida* 'Surprise® White Improved'.

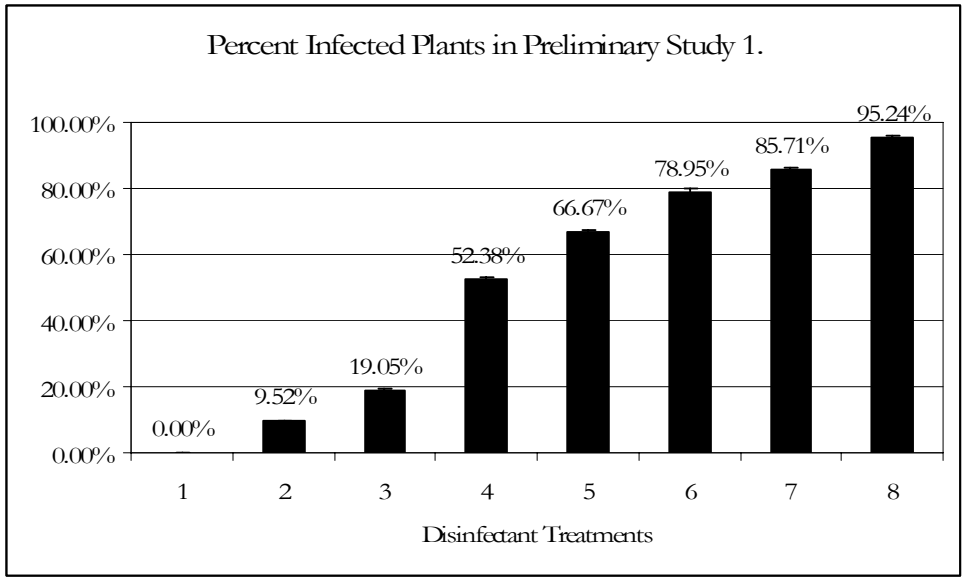

Figure 2. Percent infected plants in Preliminary Study 1. TMV-contaminated razor blades were treated for one minute with: 1 – household bleach (6% Sodium hypochlorite); 2 – flame; 3 – non-fat dry milk (20% weight/volume); 4 – Lysol® aerosol; 5 – Tri-sodium phosphate (saturated); 6 – GreenShield® (label rate); 7 – TSP substitute (sodium carbonate; saturated); 8 – water (control).

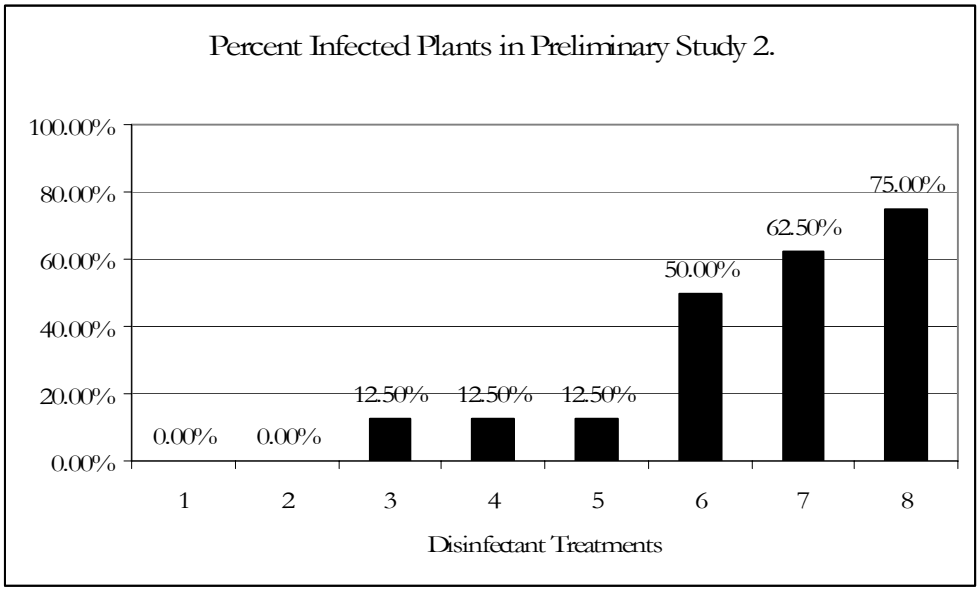

Figure 3. Percent infected plants in Preliminary Trial I. TMV-contaminated razor blades were treated for one minute with: 1 – mock (negative control) – ZeroTol® (label rate); 3 – household bleach (6% Sodium hypochlorite); 4 – GreenShield® (label rate); 5 – non-fat dry milk (20% weight/volume); 6 – Tri-sodium phosphate (saturated); 7 – TSP substitute (sodium carbonate; saturated); 8 – water (control)

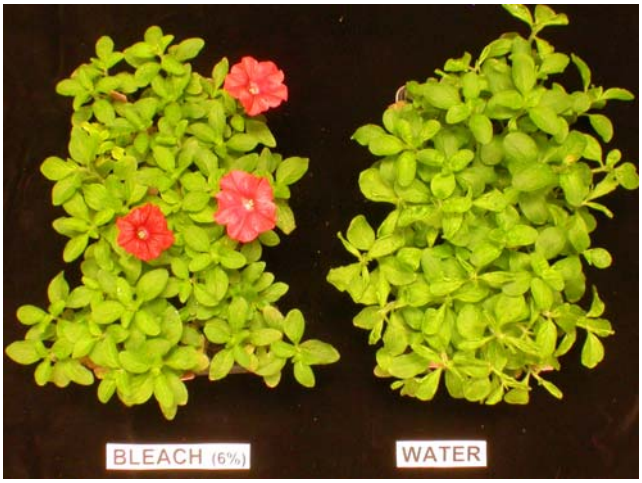

Figure 4. Bleach vs. positive control (water) plants in Preliminary Study 1, 2007.

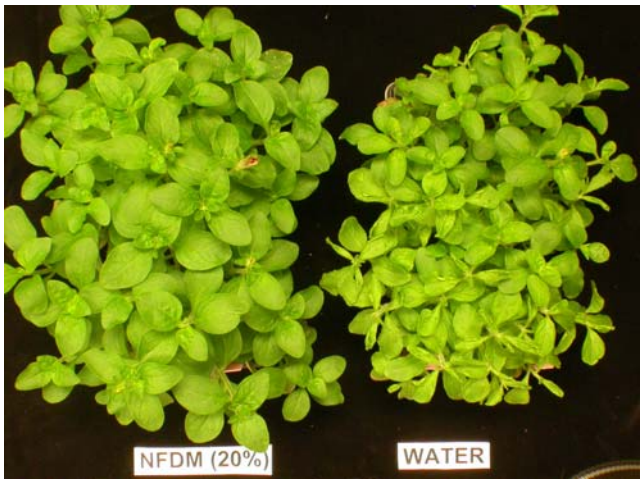

Figure 5. Non-fat dry milk vs. positive control (water) plants in Preliminary Study 1, 2007.

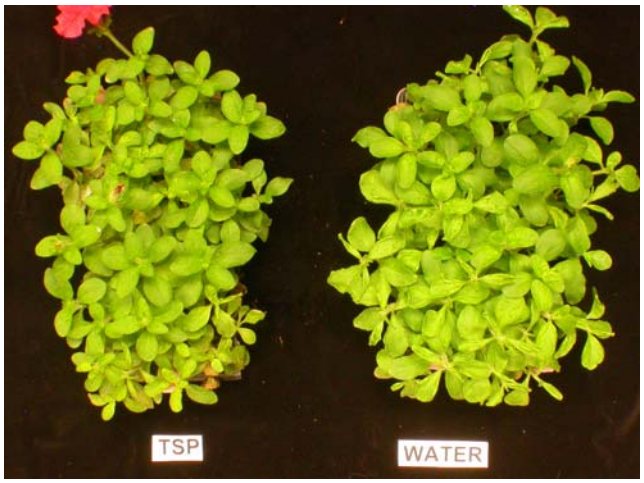

Figure 6. Tri-sodium phosphate vs. positive control (water) plants in Preliminary Study 1, 2007.

Figure 7. Plant arrangement in Phase I to prevent plants of different biological replicates from touching. Each row of plants is one biological replicate.

X				X				X				X				X
X				X				X				X				X
X				X				X				X				X
X				X				X				X				X
X				X				X				X				X
X				X				X				X				X

Figure 8. Liner tray map of plants, Phase I. Cells containing a plant are marked with an X and empty cells are left blank.

Figure 9. Plant arrangement in the greenhouse, Phase I.

Figure 10. Binder clip with razor blades aligned for contamination with TMV and disinfectant treatments, Phase I.

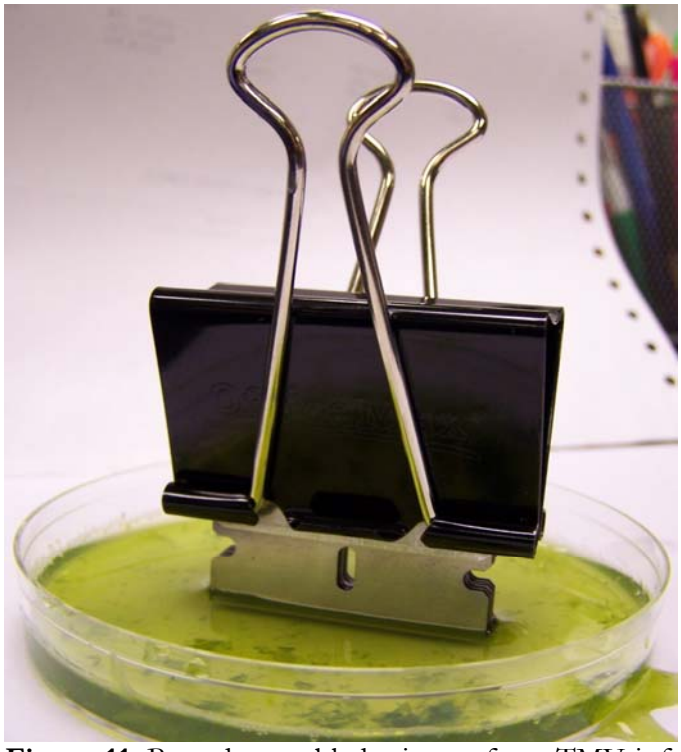

Figure 11. Bound razor blades in sap from TMV-infected petunia, Phase I.

BIBLIOGRAPHY

Albajes, R., Gullino, M.L., Van Lenteren, J.C., and Elad, Y. 1999. *Integrated Pest and Disease Management in Greenhouse Crops*. Kluwer Academic Publishers, Dordrecht.

Bettiol, W. 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginous)* in greenhouse conditions. *Crop Protection* 18:489-492.

Bockholt, S., Hellwald, K.H., and Buchenauer, H. 1988. Investigation of the influence of two quaternary ammonium compounds on some viruses and their mode of action. *Journal of Phytopathology* 127:331-340.

Brock, R.D. 1952. The use of trisodium phosphate as an inactivating agent for plant viruses. *Journal of Australian Agricultural Sciences* 18:41-43.

Chung, B.N., Kim, J.S., Cho, J.D., Cheong, S.R., and Jeong, M.I. 2007. *Tobacco mosaic virus* Detected in Vegetatively Propagated Petunia Hybrids 'Surfinia'. *Plant Pathology Journal* 23 (1): 34-36.

Copes, W.E., and Hendrix, F.F. 1996. Chemical Disinfection of Greenhouse Growing Surface Materials Contaminated with *Thielaviopsis basicola. Plant Disease* 80 (8): 885-886.

Daughtrey, M.L., and Benson, D.M. 2005. Principles of Plant Health Management for Ornamental Plants. *Annual Review of Phytopathology* 43:141-69.

Dvorak, G. 2005. Disinfection 101. The Center for Food Security and Public Health, Iowa State University. www.cfsph.iastate.edu/BRM/resources/Disinfectants/Disinfection101Feb2005.pdf

Henn, A. 2004. Tobacco Mosaic Virus. Mississippi State University Extension Service Information Sheet 1665.

Howard, R., Harding, M., Savidov, N., Lisowski, S., Burke, D., and Pugh, S. 2007. Identifying Effective Chemical Disinfectants for Use in Sanitizing Greenhouses. *Interim Progress Report II,* Alberta Professional Horticultural Growers Congress and Foundation Society.

Ingram, D.L. 1993. Landscape Plant Propagation Workbook: Unit I. General Principles of Plant Propagation. University of Florida IFAS Extension. CIR 723.

Jeffrey, D.J. 1995. Chemicals used as disinfectants: active ingredients and enhancing additives. *Revue Scientifique et technique Office International des Épizooties* 14 (1): 57-74.

Kamenova, I., and Adkins, S. 2004. Comparison of Detection Methods for a Novel Tobamovirus Isolated from Florida Hibiscus. *Plant Disease* 88 (1): 34-40.

Lesemann, D.E. 1996. Viruses recently detected in Vegetatively Propagated *Petunia*. *Acta Horticulturae* 432:88-92.

Lewandowski, D. 2005. Genus Tobamovirus, Type Species *Tobacco mosaic virus*. In *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. Ed: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., and Ball, L.A. California, USA, Elsevier, 2005. pp: 1009-1014.

Moorman, G.W. Tobacco Mosaic Virus. Cooperative Extension Service, The Pennsylvania State University Department of Plant Pathology. http://www.ppath.cas.psu.edu/extension/PLANT_DISEASE/pdf%20Flowers/Tobacco%20Mosaic%20Virus%20in%20Greenhouses.pdf

Nameth, S. 1996.Virus Disease of Greenhouse Floral Crops. The Ohio State University Extension factsheet HYG-3065-96.

Nester, M.T., Anderson, D.G., and Roberts Jr., C.E. *Microbiology, A Human Perspective*. USA, McGraw-Hill Science Engineering, 1995.

Pearce, B., Palmer, G., Bailey, A., Seebold, K., and Townsend, L. Management of Tobacco Float Systems. In *2008 Kentucky Tobacco Production Guide*. University of Kentucky ID-160. Ed: Seebold, K., and Pearch, B. pp. 12-22.

Phillipson, B., and Weekes, R. 2005. Containment of GM plant viruses being developed as gene technology vectors. www.hse.gov.uk/research/rrhtm/rr378.htm.

Pundt, L. 2003. Some Virus Diseases of Greenhouse Crops. University of Connecticut Integrated Pest Management. http://www.hort.uconn.edu/ipm/greenhs/htms/gcrpvirus.htm.

Smith, T. 2007. Cleaning and Disinfecting the Greenhouse. University of Massachusetts, Amherst Department of Plant, Soils, and Insect Sciences. www.umass.edu/umext/floriculture/fact_sheets/greenhouse_management/ghsanitz.html.

Tambascio, S. 2007. Yesterday, Today, Tomorrow. *Greenhouse Grower*, May 2007: 1-8.

Tambascio, S. 2008. Top Crops. *Greenhouse Grower*, August 2008: 34.

Williams-Woodward, J. 2000. 1999 Georgia Plant Disease Loss Estimates. Cooperative Extension Service, The University of Georgia College of Agricultural and Environmental Sciences. Special Bulletin 41-02/July 2000.