2011. Plant Management Network. This article is in the public domain.
Accepted for publication 20 December 2010. Published 21 February 2011.

A Comparison of Disinfectants to Prevent Spread of Potyviruses in Greenhouse Tomato Production

W. M. Wintermantel, USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905

Corresponding author: W. M. Wintermantel. bill.wintermantel@ars.usda.gov

Wintermantel, W. M. 2011. A comparison of disinfectants to prevent spread of potyviruses in greenhouse tomato production. Online. Plant Health Progress doi: 10.1094/PHP-2011-0221-01-RS.

Abstract

Potyviruses, transmitted by a diverse array of common aphid species, infect a broad range of vegetable crops, and can be problematic in greenhouse tomato production. Once introduced, these viruses are believed to be transmitted plant-to-plant during pruning operations, and can infect large sections of a greenhouse, resulting in significant losses in fruit quality and yield. Several methods are used for virus management in greenhouse production, including rouging of diseased plants and treatment of tools and facilities with virucides to eradicate the virus responsible. To clarify potyvirus transmission efficiency from an infected source during pruning operations, experiments were conducted using direct and serial mechanical inoculation of *Potato virus Y* (PVY) using a scalpel dipped in a suspension of PVY-infected plant sap. Tests demonstrated that both serial and direct inoculation resulted in significant PVY transmission, but that transmission rates declined after the first few plants in serial transmission. Additional tests evaluated the efficiency of two virucides, a quaternary ammonium solution and sodium hypochlorite, for virus inactivation during pruning operations using a range of concentrations and time points. Results demonstrated that 0.5% sodium hypochlorite treatment for two seconds was sufficient for virus control, and superior to treatment with quaternary ammonium solutions.

Introduction

Viruses within the genus *Potyvirus*, family *Potyviridae*, can be a significant problem for greenhouse tomato production. Two potyviruses, *Potato virus Y* (PVY) and *Tobacco etch virus* (TEV), occur with some frequency in tomato greenhouses with adjacent or nearby production of other affected crops, particularly vegetables or potato (3,4). However, limited information is available on spread and management of potyviruses in greenhouse tomato production. Potyviruses can cause symptoms of foliar mottling, puckering, and slight distortion or rugosity (10), and can be spread from plant-to-plant not only by aphid vectors, but also by mechanical transmission. Some tomato-infecting potyviruses, such as *Tobacco etch virus*, can produce fruit mottling, preventing marketability (10) and significantly reducing yields. Efforts to limit losses and prevent spread of potyviruses throughout the greenhouse can result in the grower not only removing infected plants and those plants adjacent to the infected plants, but also taking significant sections of a greenhouse out of production for a period of time, further reducing profits. Removal of infected plants is often necessary to eliminate sources of infection prior to replanting in order to prevent further infections from developing.

Potyviruses can enter greenhouses by a number of methods. Two common modes of accidental introduction include movement of viruliferous aphid vectors from nearby infected field crops or weeds into a greenhouse through vents, doors, or other points of entry. Alternatively potyviruses can enter on contaminated nursery material. Once infected plants are introduced, the viruses can spread from plant-to-plant through transmission by aphid vectors that may be present in the greenhouse. Growers have speculated that potyviruses may also spread down rows during pruning operations, but little information is available on how readily this actually occurs. Growers routinely attempt to

control mechanically transmitted viruses by removing diseased plants and those around them in an effort to reduce the source of inoculum. Additional measures include the use of chemical or heat treated tools for trimming and/or harvesting operations. Chemicals (including those referred to as virucides that eliminate viruses) can vary from sodium hypochlorite (bleach) based solutions, to dipping tools in milk, to new compounds specifically designed to inactivate viruses and other pathogens through surface sterilization (3,7). Although these methods are often used to control mechanically transmitted viruses in greenhouse operations, limited information exists on their effectiveness for potyvirus control in greenhouses. Studies were conducted to determine the ease at which potyviruses can spread in a tomato production greenhouse as a result of pruning operations, as well as the effectiveness of two common methods for treatment of tools and equipment to prevent virus spread. The purpose of these studies was three fold: (i) to determine the ability of a potyvirus to spread within rows during pruning operations; (ii) to determine if treatment of tools with virucides can reduce or eliminate within row spread of a potyvirus during pruning operations; and (iii) to determine how length and concentration of virucide treatment influences effectiveness of potyvirus control.

Virus and Inoculum Preparation

The PVY isolate used for these studies was obtained from a tomato greenhouse in southern California during a potyvirus outbreak, and was maintained at the USDA-ARS in Salinas, CA. Its identity was confirmed by sequencing of RT-PCR products obtained using potyvirus group specific primers CWC6 and CWC7 (5) targeting the NIb/CP region of the virus genome and comparison of the sequences of clones derived from RT-PCR amplicons to known sequences in GenBank. Inoculum for the experiments consisted of PVY-infected *Nicotiana benthamiana*, inoculated 3 weeks prior to the beginning of the experiment and exhibiting strong symptoms at the time of use as inoculum. *N. benthamiana* was used as a propagation host for PVY inoculum due to its ability to rapidly produce high concentrations of virus. For each inoculation, 3.5 g of symptomatic *N. benthamiana* leaves were ground in 10 ml of 100 mM sodium phosphate buffer, pH 8.0, containing 0.1% sodium sulfite, and kept on ice for the duration of inoculation. Inoculation was performed by dipping a scalpel blade in the inoculum suspension, then using the scalpel to make a diagonal slice in the stem of a tomato plant just above a leaf attachment point (node). Plants were inoculated when approximately 30 cm tall. Tomatoes (*Solanum lycopersicum*) used in all experiments were the variety VFN8 (University of California, Davis), kindly provided by Seminis Vegetable Seeds (Woodland, CA). The study consisted of two experiments, each containing five to six replicates of nine treatments with 8 plants per treatment, arranged in a randomized complete block design. Experiments were conducted sequentially in a greenhouse at the USDA-ARS in Salinas, CA.

Potyvirus Spread During Pruning Operations

To determine the ability of potyviruses to spread down a row through pruning operations, VFN8 tomato plants were inoculated with PVY using two inoculation approaches: direct inoculation and serial inoculation. Direct inoculation involved inoculation of each tomato plant with a scalpel dipped in freshly prepared ground plant sap prepared as described above, immediately prior to inoculation. Tomato seedlings were inoculated by slicing diagonally into the stem to a depth of approximately one third of the stem diameter, to provide an illustration of PVY movement in the greenhouse as a result of mechanical transmission during pruning operations. Serial inoculation was performed similarly, with the first plant inoculated as described for direct inoculation, followed by consecutive inoculation of a series of plants without cleaning of inoculation tools or dipping in fresh inoculum. This approach was designed to illustrate how many times a potyvirus can be transmitted in consecutive cuts once the virus is acquired on tools from a single infected source plant in a row, as would occur during routine greenhouse pruning operations. For each

replicate within each experiment, a negative control treatment was included consisting of an identical set of plants, sliced with a virus-free scalpel (no inoculum).

Treatment of Pruning Tools for Control of Potyviruses

A commercial quaternary ammonium virucide used for disinfection of greenhouses and associated pruning tools (5-10% alkyl dimethyl ethylbenzyl ammonium chloride and 5-10% alkyl dimethyl benzyl ammonium chloride, hereafter referred to as quaternary ammonium) was compared with chlorine bleach (5% sodium hypochlorite) for effectiveness in controlling potyvirus transmission in greenhouse tomato production (Table 1). Quaternary ammonium was used at two concentrations; 1% (standard recommended concentration) and 3%. Sodium hypochlorite (chlorine bleach) was used at 10% (1:10 dilution of 5% stock, which is the standard for bleach sterilization in laboratories and greenhouses) and 15%. The active concentrations of sodium hypochlorite in our experiments were therefore 0.5% sodium hypochlorite (considered 10% bleach solution since its concentration is only 10% of standard bleach) and 0.75% sodium hypochlorite (considered 15% bleach solution since it is 15% the concentration of standard bleach). The purpose of the times and treatments used was to compare the effectiveness of virucide treatments, as well as the length of time and type of treatment necessary for each virucide to effectively destroy potyviruses and prevent their transmission during pruning operations. Treatment times were chosen based on knowledge of current tomato greenhouse practices, and an effort to keep treatment times realistically short, yet sufficiently long to destroy virus particles. One month after inoculation, plants were scored for visible virus symptoms (*data not shown*), and samples were collected from the third compound leaf (up from the base of the plant) and tested by enzyme linked immunosorbent assay (ELISA) using the Agdia PVY detection kit (Agdia Inc., Elkhart, IN) using standard procedures as recommended by the manufacturer. The entire experiment was conducted twice, with the number of infected plants for each treatment confirmed by ELISA presented in Figure 1. Significant effects were determined through ANOVA using the GLM procedure. Least squares means were separated with the Tukey-Kramer test ($P < 0.05$) (SAS v9.2, SAS Institute Inc., Cary, NC). Both experiments consisted of 5 replications; however, the second experiment included one additional replication of the quaternary ammonium treatments. For each experiment, a negative control treatment was included consisting of an identical set of plants, sliced with a virus-free scalpel (no inoculum), as in tests comparing direct and serial transmission. Both direct and serial inoculation methods also served as untreated controls for analysis of virucide effectiveness.

Table 1. Treatments to determine transmission of *Potato virus Y* in tomato by direct and serial inoculation during pruning, and effectiveness of six control treatments in preventing virus transmission.

DI	Scalpel dipped in inoculum prior to cutting each plant (direct inoculation).
SI	Scalpel dipped in inoculum prior to cutting first plant, but used to continuously cut (inoculate) 8 consecutive plants without adding further inoculum (serial inoculation).
T1	Scalpel dipped in inoculum, then dipped 2 seconds in 10% bleach (1:10 dilution of 5% sodium hypochlorite solution) prior to cutting (inoculating) each plant.
T2	Scalpel dipped in inoculum, then dipped 15 seconds in 10% bleach solution prior to cutting (inoculating) each plant.
T3	Scalpel dipped in inoculum, then dipped 15 seconds in 15% bleach solution prior to cutting (inoculating) each plant.
T4	Scalpel dipped in inoculum, then dipped 2 seconds in 1% quaternary ammonium solution prior to cutting (inoculating) each plant.
T5	Scalpel dipped in inoculum, then dipped 15 seconds in 1% quaternary ammonium solution prior to cutting (inoculating) each plant.
T6	Scalpel dipped in inoculum, then dipped 15 seconds in 3% quaternary ammonium solution prior to cutting (inoculating) each plant.
C	No treatment, plants not exposed to virus or virucides.

Fig. 1. Comparison of PVY infection of tomato between serial and direct inoculation methods and among treatments of pruning tools with either sodium hypochlorite solutions (bleach) or quaternary ammonium solutions. DI = Direct Inoculation (blade passed through PVY solution immediately prior to cutting the stem of each tomato plant); SI = Serial Inoculation (blade passed through PVY solution immediately prior to cutting the stem of the first tomato plant, but then used to cut the next 7 plants without exposure to any additional inoculum); Treatments 1 through 6 (T1-T6) were performed using the direct inoculation method, with blade dipped in inoculum, then virucide, before cutting stem of plant.

T1 = blade dipped 2 seconds in 10% sodium hypochlorite (bleach);
T2 = blade dipped 15 seconds in 0.5% sodium hypochlorite;
T3 = blade dipped 15 seconds in 0.75% sodium hypochlorite;
T4 = blade dipped 2 seconds in 1% quaternary ammonium solution;
T5 = blade dipped 15 seconds in 1% quaternary ammonium solution;
T6 = blade dipped 15 seconds in 3% quaternary ammonium solution.

Means with the same letter are not significantly different ($P < 0.05$) based on analysis of least square means using the Tukey-Kramer method.

Plant-to-Plant Transmission of PVY is Efficient Both With Direct Transmission from an Infected Source and During Serial Trimming Operations

Results of two independent, replicated experiments indicated that direct inoculation resulted in the greatest plant-to-plant spread among all treatments (35% overall; Fig. 1). Serial inoculation resulted in slightly lower virus transmission (23%; Fig. 1), although the two treatments were not significantly different from one another with a 95% confidence interval ($P = 0.0308$). The slightly reduced serial transmission rate likely occurred because some inoculum was lost each time a new virus-free plant was cut (inoculated), and although potyviruses are mechanically transmissible, they are not as stable as some other RNA viruses affecting greenhouse tomato production, such as *Tobacco mosaic virus*, *Pepino mosaic virus*, or *Tomato bushy stunt virus* (1,8,9). Within each experimental block, plants were numbered 1 through 8 with plant 1 being the first plant inoculated in serial inoculation after passing the blade through a suspension of virus inoculum, and plant 8 the last. In direct inoculation each plant was sliced immediately after passing the blade through a suspension of virus inoculum, resulting in plants 1 through 8 having equal exposure to PVY. As expected, there was no pattern among plants numbered 1 through 8 with regard to the percent transmission to a given plant number when direct inoculation was used (Fig. 2). In contrast, percent transmission using the serial inoculation method, which most closely resembles the type of transmission that would likely occur during pruning operations, was highest in plants one and two (43% each), then decreased steadily with increasing plant numbers over the first six plants of each replication (Fig. 2). Surprisingly, higher infection percentages were observed with serial transmission to plants 7 and 8. The higher infection percentages occurred because five of 11 plants number 7 were infected by serial transmission in experiment two, resulting in the higher cumulative percent infection presented in Figure 2 (only two plants number 7 became infected in Experiment 1). Three plants number 8 were infected by serial transmission in each experiment. The ability of several plants 7 and 8 to become infected during serial transmission demonstrated mechanical transmission of PVY can still occur even after several cuts with an infected blade. No PVY infection occurred in any plants that were cut with a virus-free blade (*data not shown*). These results indicate that PVY is likely to be transmitted mechanically during greenhouse pruning operations if tools are not cleaned with antiviral agents between plants, and transmission can occur even after several cuts following exposure to virus.

Fig. 2. Comparison of percent infection between serial and direct inoculation of each of 8 plants. Plants in each block were numbered 1 through 8, with plant 1 being the first plant inoculated and plant 8 the last. Black bars indicate percent infection of each plant during serial inoculation, in which blade was dipped in inoculum prior to inoculating plant 1, and not dipped or sterilized during sequential inoculation of plants 2 through 8. Hashed bars indicate percent infection of each plant during direct inoculation, in which blade was dipped in inoculum prior to inoculation of each plant.

Results also demonstrated that mechanical (plant-to-plant) transmission of PVY during cutting operations was higher when quaternary ammonium was used than when sodium hypochlorite (bleach) was used as the antiviral agent at recommended and standard concentrations, respectively (Fig. 1; $P < 0.0001$). Plant virus research labs routinely use a 10% dilution of sodium hypochlorite (usually sold as 5 to 6%) for elimination of virus from bench tops or from pruning tools. All three bleach treatments were highly effective in eliminating potyvirus transmission (PVY was transmitted to only one plant among all 240 plants from all treatments in both experiments in which inoculation tools were exposed to bleach treatment), with transmission being less than the serial ($P = 0.0007$ for T1 and T2; $P = 0.0013$ for T3) and direct inoculation controls ($P < 0.0001$ for all 3 bleach treatments). Results with quaternary ammonium were more variable. The results clearly demonstrated that a short two second dip of cutting tools in 1% quaternary ammonium (recommended concentration) was not effective for control of PVY transmission and did not differ from untreated controls (Fig. 1). In contrast, treatment for 2 seconds with 0.5% sodium hypochlorite completely eliminated PVY transmission. In fact, levels of transmission when tools were treated for 2 seconds with the 1% quaternary ammonium overall were not significantly different from the direct inoculation or serial inoculation control treatments (Fig. 1; $P = 0.6231$ with direct inoculation and $P = 0.1883$ for serial inoculation). There was considerable variation among replications for this treatment (T4). Treatments 5 and 6 confirmed that tools required longer treatment with the quaternary ammonium solution for activity against PVY than with sodium hypochlorite. The longer quaternary ammonium treatments (T5 and T6) were more effective for virus decontamination of tools, but even exposure of cutting tools to one and three percent quaternary ammonium for 15 seconds each resulted in several infected plants (Table 3, Fig. 1). Longer quaternary ammonium treatment times (T5 and T6) resulted in improved control compared with the 2-second (T4) treatment period ($P = 0.0002$ to 0.0021). Manufacturers of quaternary ammonium solutions usually recommend a longer treatment period; however, longer periods are not always convenient during pruning operations. Although waiting 15 seconds between each plant would considerably lengthen the time required for pruning operations, many programs accommodate this recommendation by

using two sets of tools, with one set soaking while the other is in use. Consequently, with longer treatment periods the quaternary ammonium solution may result in an acceptable level of control, although likely it would not eliminate all potyvirus transmissions.

In contrast, if tools are treated with dilute (10%) bleach solution as in these experiments, it appears that control of mechanically transmitted PVY and likely other potyviruses, which are physically similar to PVY, will improve tremendously. Even dipping cutting tools in a 10% bleach solution for as little as 2 seconds was highly effective in eliminating PVY transmission in these studies (Table 2, Fig. 1). Our recommendation to the industry would be to either convert to bleach treatment of pruning equipment or consider treating pruning tools for much longer periods of time between plants with the quaternary ammonium solutions. Bleach may be more cost-effective than quaternary ammonium solutions, which are usually purchased as commercial products from various vendors; however, it is possible that bleach may result in corrosion of equipment over time (2). This could be remedied by rinsing equipment with water periodically during trimming operations and following completion of trimming. Additionally, it has been recommended that drying of pruning knives after disinfection and rinsing can reduce the corrosiveness of sodium hypochlorite solutions (2). Interestingly, the results presented herein for potyvirus control are consistent with those of Lewandowski et al. (7) who evaluated performance of several commercial and non-commercial treatments for control of tobamoviruses in floriculture production. Lewandowski and colleagues demonstrated that sodium hypochlorite treatment was far superior to quaternary ammonium treatment for tobamovirus control. Tobamoviruses are more readily transmissible by pruning operations, and are much more stable than potyviruses. Similarly, Copes and Hendrix (6) demonstrated that sodium hypochlorite treatment significantly reduced viability of fungal spores, whereas ammonium compounds were less effective. Although the risk of corrosive issues on pruning tools remains a concern, these studies demonstrate that sodium hypochlorite treatment remains one of the most reliable means to virtually eliminate transmission of viral agents in greenhouses.

Acknowledgments

Special thanks to Art Cortez for assistance with greenhouse experimentation, and to Dr. Kelley Richardson for statistical analysis. This work was supported by a grant from the California Tomato Commission.

Literature Cited

1. Broadbent, L., and Fletcher, J. T. 1963. The epidemiology of tomato mosaic IV. Persistence of virus on clothing and glasshouse structures. *Ann. Appl. Biol.* 52:233-241.
2. Celar, F., Nevenka, V., Kosmelj, K., and Gril, T. 2007. Evaluating the efficacy, corrosivity and phytotoxicity of some disinfectants against *Erwinia amylovora* (Burrill) Winslow et al. Using a new statistical measure. *J. Plant Dis. Protect.* 114:49-53.
3. Cerkauskas, R. 2005. Tomato Diseases: *Potato virus Y*. Publ. 05-625. AVRDC, The World Vegetable Center, Shanhua, Taiwan.
4. Cerkauskas, R. 2005. Tomato Diseases: *Tobacco etch virus*. Publ. 05-640. AVRDC, The World Vegetable Center, Shanhua, Taiwan.
5. Collmer, C. W., Marston, M. F., Albert, S. M., Bajaj, S., Maville, H. A., Ruuska, S. E., Vesely, E. J., and Kyle, M. M. 1996. The nucleotide sequence of the coat protein gene and 3' untranslated region of Azuki bean mosaic potyvirus, a member of the Bean common mosaic virus subgroup. *Mol. Plant Microb. Interact.* 9:758-761.
6. Copes, W. E. and Hendrix, F. F. 1996. Chemical disinfection of greenhouse growing surface materials contaminated with *Thielaviopsis basicola*. *Plant Dis.* 80:885-886.
7. Lewandowski, D. J., Hayes, A. J., and Adkins, S. 2010. Surprising results from a search for effective disinfectants for *Tobacco mosaic virus*-contaminated tools. *Plant Dis.* 94:542-550.

8. Ling, K. S. 2008. *Pepino mosaic virus* on tomato seed: Virus location and mechanical transmission. *Plant Dis.* 92:1701-1705.
9. Martelli, G. P., Gallitelli, D., and Russo, M. 1988. Tombusviruses. Pages 13-72 in: *The Plant Viruses*, Vol. 3. R. Koenig, ed. Plenum, New York, NY.
10. Zitter, T. A. 1991. *Potato virus Y* and *Tobacco etch virus*. Pages 36-38 in: *Compendium of Tomato Diseases*. J. B. Jones, J. P. Jones, R. E. Stall, and T. A. Zitter, eds. American Phytopathological Society, St. Paul, MN.