Getting to 100%: a two step method for orchid virus disinfection (CymMV, ORSV) of repotting tools

Two step procedure for orchid virus disinfection of cutting and repotting tools with NaOH and Virkon S

The title of this article evokes a key, but often missed question: how effective is your process for disinfecting your cutting tools for orchid viruses? We have used bleach, flaming, and Physan 20 for years, and I assumed that they were completely effective in removing virus particles from our shears, Exacto blades, pots, and working surfaces. A great expectation that I have come to realize was probably very inaccurate.

Research on Common Orchid Virus Disinfection Methods

In a talk at the San Francisco Orchid Society, Dr. Kay Klausing, a molecular biologist and orchid grower, reviewed scientific studies of the common disinfection methods for orchid viruses, specifically Cymbidium Mosaic Virus (CymMV) and Odontoglossum Ringspot Virus (ORSV). The research examined numerous common approaches, including flaming tools, and soaking in saturated TSP, diluted NaOH (Iye), Physan 20, and isopropanol.

Stunningly, none of these were found to be 100% effective for the inactivation of orchid viruses; most were in the 90-99% range, with Physan 20 and isopropanol being largely ineffective. One factor was contact time; in the research, all were assumed to be less ten minutes. No contact time for flaming was provided, and there are wide range of approaches, from heating metal cutting surfaces red hot to casually passing blades through a flame.

While it would be excellent to have a more detailed study, these findings were certainly sufficient to have us rethink our orchid virus disinfection protocols. Although it is possible to debate the details of each method, it certainly appeared that we were only in the 90-99% range for orchid virus disinfection. For our orchid growing, that's not good enough! I'd like my cutting tools to be 100% free of orchid viruses when I work on any plant. We take orchid viruses very seriously and have tested our entire collection for CymMV and ORSV; learn more in this article.

The Unique Resistance of Orchid Viruses to Disinfection

Orchid viruses are exceptionally tenacious at persisting in the environment, and as a result, extra effort is required to reach 100% deactivation of ALL virus particles.

Initially, I was interested in the possibility of using ultraviolet (UV) light as part of our orchid virus disinfection (and general sanitation) protocol. It is widely used in laboratories, ambulances, and spa/medical sterilization. With a little investigation, I realized the unique nature of plant viruses compared to common human pathogens. A <u>table of UV irradiation dosages</u> has been compiled for several dozen common pathogens.

For reference, the UV irradiation dosage to reach 99% disinfection of common bacteria is 3,500-22,000 μ Ws/cm2. Anthrax spores were a notable high of 46,200 μ Ws/cm2. Influenza and polio virus requires a UV dosage of 6,600 μ Ws/cm2 for 99% elimination.

Included on the list is Tobacco Mosaic Virus (TMV). ORSV is part of the TMV family of viruses. We can draw similar assumptions for the closely related ORSV. Remarkably, TMV requires a dosage of 440,000 μ Ws/cm2 for 99% disinfection — nearly 10x that of anthrax spores and 100x that of common bacteria. The only pathogens with a comparable level of required UV dosage were a few types of mold, including *Aspergillus niger* and *Risopus nigricans*.

(Despite the inclusion of "mosaic" in the name, CymMV is in a different family of virus — Alphaflexiviridae, including the genus of *Potexvirus*. ORSV is in the family of Virgaviridae and one of the *Tobamovirus*.) Suffice to say, the requirements for disinfection of orchid viruses are in a league of their own, far greater than for any other pathogens that we might commonly encounter in our home and work environments.

Getting to 100% Disinfection of Orchid Viruses

Based upon the available research, Dr. Klausing recommended a two step process for 100% orchid virus disinfection of tools. The first step is "cleaning" at elevated pH and the second step involves oxidation of the virus RNA.

NaOH for orchid virus disinfection: step 1

For the first step, he suggests using full strength bleach at elevated pH, saturated TSP, or 1M (one molar) NaOH. For the second step, he recommend using bleach at its natural (off-the-shelf) pH or a disinfectant called Virkon S. In his protocol, he sprays his tools with 1M NaOH and Virkon S, and allows them to hang on a rack for each stage for a minimum of five minutes, alternating with a water rinse between stages. Many of our orchids are indoors, and spraying lye or bleach around our bathroom isn't very practical. We also often do a lot of orchid mounting and repotting in the evenings, on our "indoor potting bench," a.k.a. a spare table with a bright overhead light. So we needed a way to be able to expediently disinfect our tools in the house.

My solution? I purchased a deep plastic wash basin and six 500ml glass beakers (see top photo). Instead of spraying, I soak the shears in the beakers of NaOH and Virkon S.

First, I wash the shears to remove any particulate matter; I usually spray/scrub as needed with Physan 20 in the sink. Second, I soak the shears in 1M NaOH, for five minutes (or more). A one molar solution of NaOH is produced by dissolving NaOH (Iye) granules at a concentration of 40g/L. 300mL of solution adequately covers my shears. I purchased a one pound bag of food-grade lye and weigh the appropriate quantity with a small kitchen scale.

Next, I rinse the shears in the sink. They are then immersed in a 2% solution of Virkon S disinfectant for another five minutes.

Virkon S for orchid virus disinfection: step 2

Virkon S is widely used in agriculture and veterinary applications, and available in small to enormous quantities (think washing down a dairy operation). The more you buy, the cheaper it gets. However, you can buy a small container of pre-measured tablets for about \$20, which lasts me for about 6-8 months. One tablet in 250mL of water produces a 2% solution. The solution lasts about 7-10 days, fading from bright yellow. I replace the solution weekly, and always before a large repotting project.

Finally, I rinse the shears again, and place them in an empty beaker to dry. Unlike flaming, I have not noticed any degradation of the blade or rusting from the NaOH and Virkon S disinfection process. We have at least seven shears, so there is always a clean pair available while others are soaking after use.

There you have it! With one order to Amazon.com for the <u>deep dish basin</u>, <u>beakers</u>, and <u>NaOH</u>, plus the (cheaper) purchase of the <u>Virkon S bottle</u> from an online agriculture supply company, I assembled a 100% orchid virus disinfection station for our cutting tools. Of course, you can use any basin or glass bottles for this purpose. I chose the beakers since they had pre-measured lines for the amount of water to add for the NaOH and Virkon S solutions, and they were the right height for our shears.

Preventing Spread of Orchid Viruses when Repotting

To create a clean surface for each plant when we repot our orchids, we use a 2" deep plastic tray lined with <u>heavy duty butcher (freezer) paper</u>. A bulk roll is inexpensive, and my husband pre-cuts the sheets with a paper cutter so we just quickly pull a new sheet for each plant while working. The butcher paper is very durable and resistant to water, with one side having a waxy coating. We often rinse orchids when

repotting and I find that newspaper soaks through layers, becoming inky and potentially distributing pathogens through quite a stack of paper. It is easy to roll up a pile of bark in the (strong) butcher paper, even from a 1-2 gallon *Cymbidium* pot, and dump it into the compost bin fully wrapped up and contained. I do not stack the sheets of butcher paper, but place a new sheet on the bench/tray after the used sheet has been removed. This way, it is assuredly a clean surface for the next plant, a separate layer over the surface of potting bench (or potting tray when indoors).

We disinfect the surface of the outdoor potting bench and indoor plastic tray with 100% bleach, after washing away any particulate matter. Our outdoor potting bench is homemade with a galvanized wire mesh top on a wood frame — no problems with using water and bleach to thoroughly disinfect the metal mesh at least after each day of use, or, if necessary, after handling a diseased orchid.

We soak our plastic pots in a 30% bleach solution for 3 days, after thoroughly removing any organic matter (brush/scrub with soap and water). While the one step process for using bleach to disinfect cutting tools was found to be less than 100% effective for eliminating viruses, allowing the pots to soak for an extended period of time compensates.

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