A COMPREHENSIVE LITERATURE REVIEW

TUMI Genomics

TOOL STERILIZATION TO PREVENT VIROID TRANSMISSION

To determine the most effective method of tool sterilization to prevent mechanical transmission of Hop Latent Viroid, we conducted a comprehensive literature review of studies investigating viroid disinfection treatments.

02

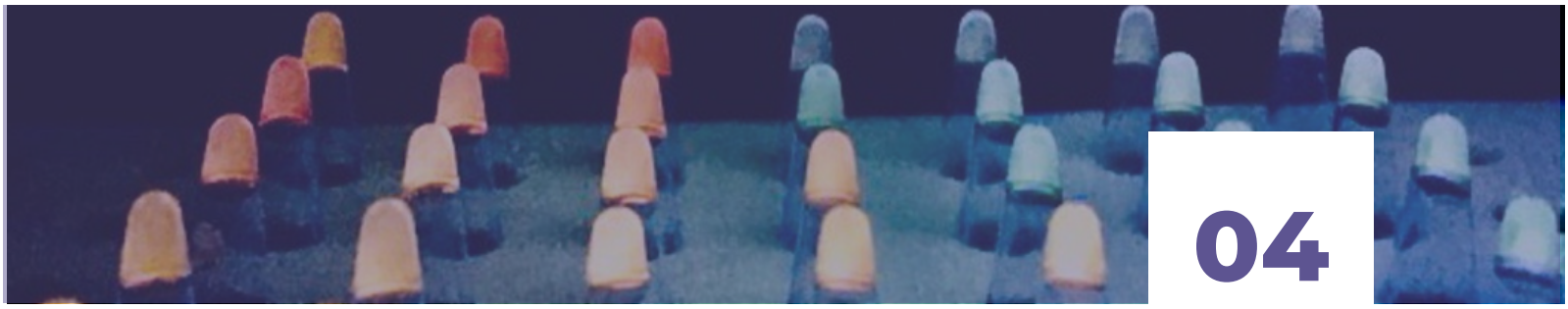
Summary

Viroids are small infectious agents that are pathogenic in plants. In contrast to viruses and other pathogens, viroids do not contain a protein or lipid component and are instead composed entirely of circular, structured RNA. RNA, similar to DNA, stores the genetic information needed for the viroid to highjack the plant's metabolism for replication. Viroid infection can drastically harm host plants. In the cannabis industry Hop Latent Viroid (HLVd) has become widespread and is causing dramatic economic losses due to reduced flower size and cannabinoid content in infected plants. HLVd, like most viroids, is spread mainly via mechanical transmission by contaminated cutting tools. Therefore, sterilization of cutting tools and equipment between each plant is critical for limiting spread within a facility, especially since infected plants can appear asymptomatic. However, not all sterilization techniques have the same efficiency against HLVd. Some sterilization solutions that are effective against bacterial or fungal pathogens do not destroy viroids. The purpose of this review was to conduct a comprehensive literature review of studies investigating viroid disinfection treatments to determine the most effective method of tool sterilization to prevent mechanical transmission of Hop Latent Viroid in plants.

03

FIFTY-THREE DIFFERENT CHEMICALS/TREATMENTS WERE EVALUATED

in this review for the ability to prevent viroid transmission in the ten referenced studies. Among these, five tested treatments showed 100% effectiveness at viroid deactivation. While most of these chemicals are shown to be effective against specific viroids (Table 1), one chemical, household bleach (sodium hypochlorite - NaClO), shows broad effectiveness across numerous studies, different plant species and multiple viroids.



Based on this observation, we strongly recommend the use of 10-20% household bleach (0.5%-1% sodium hypochlorite) for at least 60 seconds to disinfect cutting tools, equipment and surfaces in order to limit the spread of Hop Latent Viroid in cannabis grow facilities.

Bleach is also highly effective for deactivation of most other cannabis pathogens including, fungus, mold, oomycetes, bacteria.

Careful evaluation of conditions applied in each referenced study indicates that bleach is an effective disinfectant when used at a range of 10% to 20% concentration and for various incubation times. This quality provides flexibility and room for human error when employed as a broad disinfection SOP (Standard Operating Procedure) in large facilities. Because the effective concentration in dilute bleach fluctuates over time, mixing a fresh bleach solution at the start of each day or when the solution becomes saturated with plant material is recommended. However, studies indicate that 20% bleach solutions can remain effective for up to 30 days at room temperature (Li et. al., 2015) allowing for flexibility.

Several common disinfection practices are not effective at viroid decontamination, including alcohol and heat treatment, or even the combination of these two.

It should be noted that several common disinfection practices are not broadly effective at viroid decontamination. While alcohol (ethanol or isopropanol) can be effective at deactivating other plant pathogens, viroids are not deactivated by alcohol, and treatment of cutting tools with only alcohol may increase transmission of viroid infection (Table 4 and Matsuura et. al., 2010). Viroids also show tolerance to heat treatment in multiple studies. Infectiousness persisted following alcohol dip and flaming, propane flame treatment and prolonged heat incubation of contaminated tools (Table 2 and Table 3). Therefore, we recommend not relying solely on heat treatment as a regular disinfection practice for prevention of viroid transmission.

TOOL STERILIZATION RECOMMENDATION

05

To prevent mechanical transmission of viroids in cannabis facilities, a dilute bleach solution should be used to disinfect tools, surfaces and gloved hands between plants.

To create a 10% dilute bleach solution mix:

1 part household bleach (>5% active ingredient - sodium hypochlorite)
9 parts water

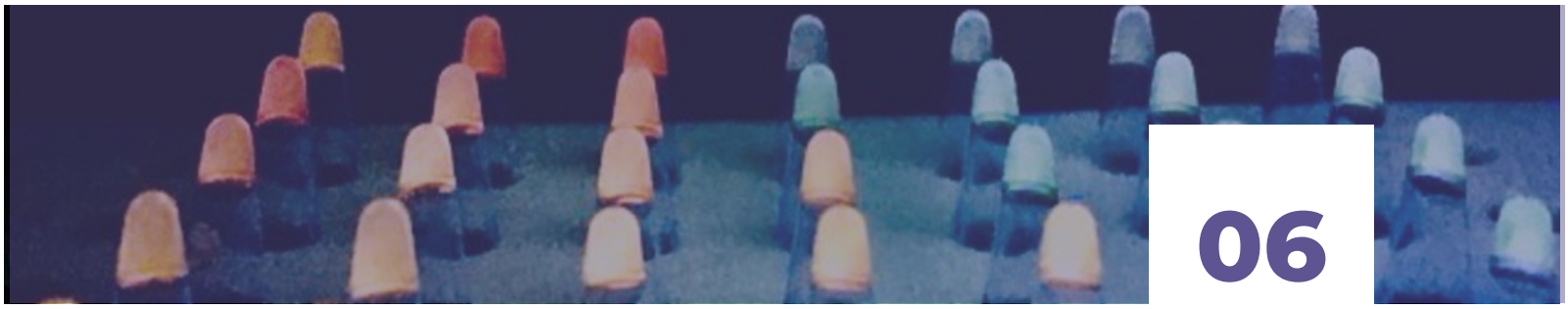
To create a 20% dilute bleach solution mix:

1 part household bleach (>5% active ingredient - sodium hypochlorite)
4 parts water

Tools should remain in the bleach solution for 60 seconds

All tools should remain immersed in the bleach solution for **60 seconds or more** to allow complete deactivation. Decontaminated tools can be rinsed in clean water prior to use. DO NOT mix bleach and alcohol as this creates toxic gas. Bleach is corrosive to metal tools so prolonged incubation is not recommended. Bleach should be stored in air-tight plastic containers and kept in a cool (500C – 700F), dry area away from sunlight. Bleach exposed to light or temperatures above 700F degrades faster. The bleach solution should be mixed fresh at the start of each day and replaced if the solution becomes saturated with plant material. Dilute bleach in a spray bottle can be used periodically to disinfect gloved hands.

TUMI Genomics



Bleach should not be stored with incompatible chemicals such as strong acids, ammonia, or alcohol. The shelf life of bleach when stored correctly is about six months. Old bleach solutions can be disposed off down the drain with ample amounts of water.

Literature Summary Data

The tables below summarize results from published scientific reports investigating the efficacy of various chemicals and treatments for removal of viroid contamination from tools. Only studies where effectiveness was determined by inoculation of clean plants with sterilized tools are included in this summary. Tables indicate the concentration(s) of each chemical used in the studies, details of the treatment applied and the percent transmissibility that remained after disinfection.

THE SOLUTIONS

Eliminates Viroid Transmission

- House hold bleach (5%-25%)
- 2%-37% formaldehyde + 2% sodium hydroxide
- 2%-3% Menno Florase (9% w/v benzoic acid)
- 2% Virkon S (20.4% potassium peroxy-monosulfate, 1.5% sodium chloride)"
- NaOH 0.5% pH 13

To create a 20% bleach solution mix:

- 1 part household bleach
- 4 parts water

Possibly Eliminates Viroid Transmission

- 95%-96% alcohol followed by flame
- 20%-100% fresh or powdered milk
- 6%-23% Hydrogen peroxide

Does Not Eliminate Viroid Transmission

- 0.5%-1% Virkon S
- 1%-5% formaldehyde
- 1%-10% Bromodine
- 2% detergent
- 1% or 10% Triodine
- 1% Roccal
- 0.1%-10% sodium hydroxyide
- 2%-10% trisodium hypochlorite
- 70%-95% ethyl alcohol
- Diesel fuel
- 20% Phisohex
- 3% formalin
- 10% Borax
- 1%-50% Lysol
- Vinegar/oil/water (4:1/3:12)
- 20% diethyl ether
- 2% trisodium orthophosphate
- Incyte
- Alcide LD
- Exspor
- 0.781% Octave
- 1% MENNOTER forte
- 0.52% GREENSHIELD
- 0.977% StorOx
- 0.5%- 1% Virkon S
- Electrolyzed acid water
- 0.1 N hydrochloric acid
- 70% isopropanol
- 2% sodium hydroxide + 2% formaldehyde
- Chlorine Dioxide 3ppm-15ppm
- "NaCO3 0.5%
- pH 11"
- NaHCO3 pH 8.15

Table 1: Highly effective solutions/treatments for viroid decontamination

Note that household bleach was 100% effective at stopping viroid transmission in every study for every tested viroid.

Solution/ Treatment	Treatment Details	Transmissibility following treatment	Viroids tested	References
House hold bleach (5%-25%) NOTE: Concentrations below 3% were not fully effective in numerous studies	10 seconds for more in bleach solution **(60 seconds recommended)**	Varied, but treatment 0% infection rate in all studies tested	ASBVd, CEVd, PSTVd, TCDVd, HSV, CSVd	Thi Thu (2018), Desjardins et. al (1987), Garnsey and Whidden (1971), Roistacher et at. (1969), Sigh et. al. (1989), Li et. al (2015), Mackie et. al (2015), Matsuura et. al. (2010), Singh et. al (1989)
2%-37% formaldehyde + 2% sodium hydroxide	Razor blade dipped in viroid extract and then used to slash seedling	0% infection rate (12 plants tested)	ASVBd	Desjardins et. al (1987), Garnsey and Whidden (1971)
2%-3% Menno Florase (9% w/v benzoic acid)	10-60 second incubation	0% infection rate (12 plants tested)	PSTVd	Timmermann et. al. (2001)
2% Virkon S (20.4% potassium peroxymonosulfate, 1.5% sodium chloride)	10-60 second dip	0% infection rate (27 plants tested)	PSTVd	Li et. al (2015)
NaOH 0.5% pH 13	15 minute exposure time	0% infection rate – 8 plants tested	TCDVd	Thi Thu (2018)

Table 2: Solutions/treatments with conflicting evidence of effectiveness for viroid decontamination

Solution/ Treatment	Effective treatment details	Ineffective treatment details	Viroids tested	References
95%-96% alcohol followed by flame	TCDVd- 0% infection rate 2-3 second dip followed by flame (8 plants tested)	CEVd- 95%-100% infection rate after 2-3 second dip followed by flame (34 plants tested, two studies)	TCDVd, PSTVd, CEVd	Thi Thu (2018), Garnsey and Shidden (1971), Roistacher et at. 1969
20%-100% fresh or powdered milk	PSTVd- 0% infectivity after 1 minute incubation – 10 plants tested (Mackie et. al.)	PSTVD and CEVD- 11%-38% infectivity rate after 2-60 seconds incubation with 20%-100% powdered milk and 7% infection rate with 100% fresh milk- 16 plants tested (CEVd)	CEVd, PSTVd	Garnsey and Shidden (1971), Li et. al. (2015), Mackie et. al. (2015)
6%-23% Hydrogen peroxide	ASVBd- 0% infectivity after dip in solution- 12 plants tested	PSTVd – Various level of infectivity in combination with other acids (see Bioside, Sanidate, Octave and Vortexx in Table 3)	ASVBd, PSTVd	Desjardins et. al (1987), Li et. al. (2015)

Table 3: Physical treatments that are not effective at viroid decontamination

Treatment	Treatment Details	Transmissibility following treatment	Viroid(s) tested	References
Flame-propane torch (4-6 seconds)	Six seconds of flame resulted in average temperature of 2220C (4320F)	61% infection rate (36 plants tested)	CEVd	Roistacher et at. 1969
Heat treatment	10 minutes at 1400C (2840F) and 10 minutes at 1000C (2120F) NOTE: 1600C (3200C) for 10 minutes was effective.	900C -1400C (1940F-2840F) infection rate ranged between 7% and 61%.	HSV, CSVd	Takahashi and Yaguchi (1984), Hollings and Stone (1973)
Ultraviolet Radiation	10-30 minute treatment	17%-38% infection rate (29 plants tested per condition)	CSVd	Hollings and Stone (1973)
Sonication	5-15 minutes	30% infection rate (29 plants tested per condition)*	CSVd	Hollings and Stone (1973)

*Infection rate of untreated ~38%

Table 4: Chemical treatments that are not effective at viroid contamination

Solution/ Treatment	Treatment Details	Study results	Viroids tested	References
0.5%-1% Virkon S	2-60 second dip	33%-100% infection rate (67 plants tested- two studies)	PSTVd	Mackie et. al (2015), Li et. al (2015)
1%-5% formaldehyde	2-30 second dip in chemical	12.5%-81% infection rate (278 plants tested- Both studies combined)	CEVd, CSVd	Garnsey and Shidden (1971), Hollings and Stone (1973)
1%-10% Bromodine	2-3 second dip or 2 minute dip (rinsed)	38% infection rate (24 plants tested). Note- 10% concentration for 2 minutes stopped transmission (8 plants tested).	CEVd	Garnsey and Shidden (1971)
2% detergent	2-3 second dip	70% infect rate (20 plants tested).	CEVd	Garnsey and Shidden (1971)
1% or 10% Triodine	2-3 second dip	75% infection rate (16 plants tested)	CEVd	Garnsey and Shidden (1971)
1% Roccal	2-3 second dip	75% infection rate (8 plants tested)	CEVd	Garnsey and Shidden (1971)

Table 4: Chemical treatments that are not effective at viroid contamination

Solution/ Treatment	Treatment Details	Study results	Viroids tested	References
0.1%-10% sodium hydroxyide	2-3 second dip, 5 minute or 10 minute treatment	54% infection rate for 2-3 second dip (28 plants tested), 0% infection rate for 5-10 minute incubation (17 plants tested- two studies)	CEVd, HSV	Garnsey and Shidden (1971) Takahashi and Yaguchi (1984)
2%-10% trisodium hypochlorite	Several seconds	25% (139 plants tested, two studies)	CEVd	Roistacher et at. 1969
70%-95% ethyl alcohol	1-60 second dip	65%-100% infection rate (30 plants tested, two studies)	CEVd, PSTVd	Roistacher et. al. (1969), Mackie et. al (2015)
Diesel fuel	2-3 second dip	100% infection rate (8 plants tested)	CEVd	Garnsey and Shidden (1971)
20% Phisohex	Several seconds	29% infection rate (14 plants tested)	CEVd	Roistacher et. al. (1969)
3% formalin	Several seconds	50% infection rate (14 plants tested)	CEVd	Roistacher et. al. (1969)
10% Borax	Several seconds	64% infection rate (14 plants tested)	CEVd	Roistacher et. al. (1969)
1%-50% Lysol	2-60 second dip	22%-100% infectivity rate (41 plants tested, two studies)	CEVd, PSTVd	Roistacher et. al. (1969), Li et. al (2015)
Vinegar/oil/water (4:1/3:12)	Several seconds	100% infection rate (9 plants tested)	CEVd	Roistacher et. al. (1969)

Table 4: Chemical treatments that are not effective at viroid contamination

Solution/ Treatment	Treatment Details	Study results	Viroids tested	References
20% diethyl ether	2-3 second dip	0.5% infection rate (18 plants tested)	CSVd	Hollings and Stone (1973)
2% trisodium orthophosphate	2-3 second dip	25% (139 plants tested)	CSVd	Hollings and Stone (1973)
Incyte	Various times depending on equipment	100% infection rate (11 plants tested)	PSTVd	Singh et. al (1989)
Alcide LD	Various times depending on equipment	Between 5-20 plants tested, variable infectivity depending on equipment	PSTVd	Singh et. al (1989)
Exspor	Various times depending on equipment	Between 5-20 plants tested, variable infectivity depending on equipment	PSTVd	Singh et. al (1989)
0.781% Octave	10-60 second dip	18.5% infectivity (27 plants tested)	PSTVd	Li et. al. (2015)
1% MENNOTER forte	10-60 second dip	18.5% infectivity (27 plants tested)	PSTVd	Li et. al. (2015)
0.52% GREENSHIELD	10-60 second dip	26% infectivity (27 plants tested)	PSTVd	Li et. al. (2015)

Table 4: Chemical treatments that are not effective at viroid contamination

Solution/ Treatment	Treatment Details	Study results	Viroids tested	References
0.977% StorOx	10-60 second dip	26% infectivity (27 plants tested)	PSTVd	Li et. al. (2015)
0.5%- 1% Virkon S	10-60 second dip	37% - 67% infectivity (18-27 plants tested)	PSTVd	Li et. al. (2015), Mackie et. al. (2015)
0.4% KleenGrow	10-60 second dip	44% infectivity rate (18 plants tested)	PSTVd	Li et. al. (2015)
0.11% Greenhouse Guardian	10-60 second dip	41% infectivity rate (27 plants tested)	PSTVd	Li et. al. (2015)
0.195% Vortexx	10-60 second dip	50% infectivity (18 plants tested)	PSTVd	Li et. al. (2015)
0.078% BioSide	10-60 second dip	56% infectivity rate (27 plants tested)	PSTVd	Li et. al. (2015)
0.382% SaniDate	10-60 second dip	63% infectivity rate (27 plants tested)	PSTVd	Li et. al. (2015)
0.1% DES-O-GERM	10-60 second dip	67% infectivity rate (18 plants tested)	PSTVd	Li et. al. (2015)
2.5%- 10% trisodium phosphate	2-60 second dip	16.7%-70% infection rate (69 plants tested, 3 studies)	TCDVd, PSTVd, CEVd	Matsuura et. al. (2010), Li et. al. (2015), Garnsey and Shidden (1971)

Table 4: Chemical treatments that are not effective at viroid contamination

Solution/ Treatment	Treatment Details	Study results	Viroids tested	References
10% FARMcleanse	1 minute dip	100% infection rate (10 plants tested)	PSTVd	Mackie et. al. (2015)
Electrolyzed acid water	15 second dip	75% infection rate (48 plants tested)	TCDVd	Matsuura et. al. (2010)
0.1 N hydrochloric acid	15 second dip	62.5% infection rate (48 plants tested)	TCDVd	Matsuura et. al. (2010)
70% isopropanol	15 second dip	95.8% infection rate (48 plants tested) -NOTE- water dip alone resulted in 78.1% infection rate.	TCDVd	Matsuura et. al. (2010)
2% sodium hydroxide + 2% formaldehyde	15 second dip	4.2% infection rate (48 plants tested)	TCDVd	Matsuura et. al. (2010)
Chlorine Dioxide 3ppm-15ppm	15 minute exposure time	100% infection rate (36 total plants tested)	TCDVd	Thi Thu (2018)
NaCO3 0.5% pH 11	15 minute exposure time	50% infection rate (8 plants tested)	TCDVd	Thi Thu (2018)
NaHCO3 pH 8.15	15 minute exposure time	50% infection rate (8 plants tested)	TCDVd	Thi Thu (2018)

References

Desjardins, P.R., Saski, P.J., Drake, R.J., 1987. Chemical inactivation of avocado sunblotch viroid on pruning and propagation tools. Calif. Avocado Soc. 1987 Yearbook. 71,259 262.

Garnsey, S.M., Whidden, R., 1971. Decontamination treatments to reduce the spread of citrus exocortis virus (CEV) by contaminated tools. Proc. Fla. State Hort. Soc. 84,63 67.

Hollings, M., Stone, O.M., 1973. Some properties of chrysanthemum stunt, a virus with the characterization of an uncoated ribonucleic acid. Ann. Appl. Biol. 74, 333 348.

Li, R., Baysal-Gurel, F., Abdo, Z., Miller, S.A., Ling, K.S., 2015. Evaluation of disinfectants to prevent mechanical transmission of viruses and a viroid in greenhouse tomato production.Virology J. 12, 5.

Mackie, A.E., Coutts, B.A., Barbetti, M.J., Rodoni, B.C., McKirdy, S.J., Jones, R.A.C., 2015. Potato spindle tuber viroid: stability on common surfaces and inactivation with disinfectants. Plant Dis. 99, 770 775.

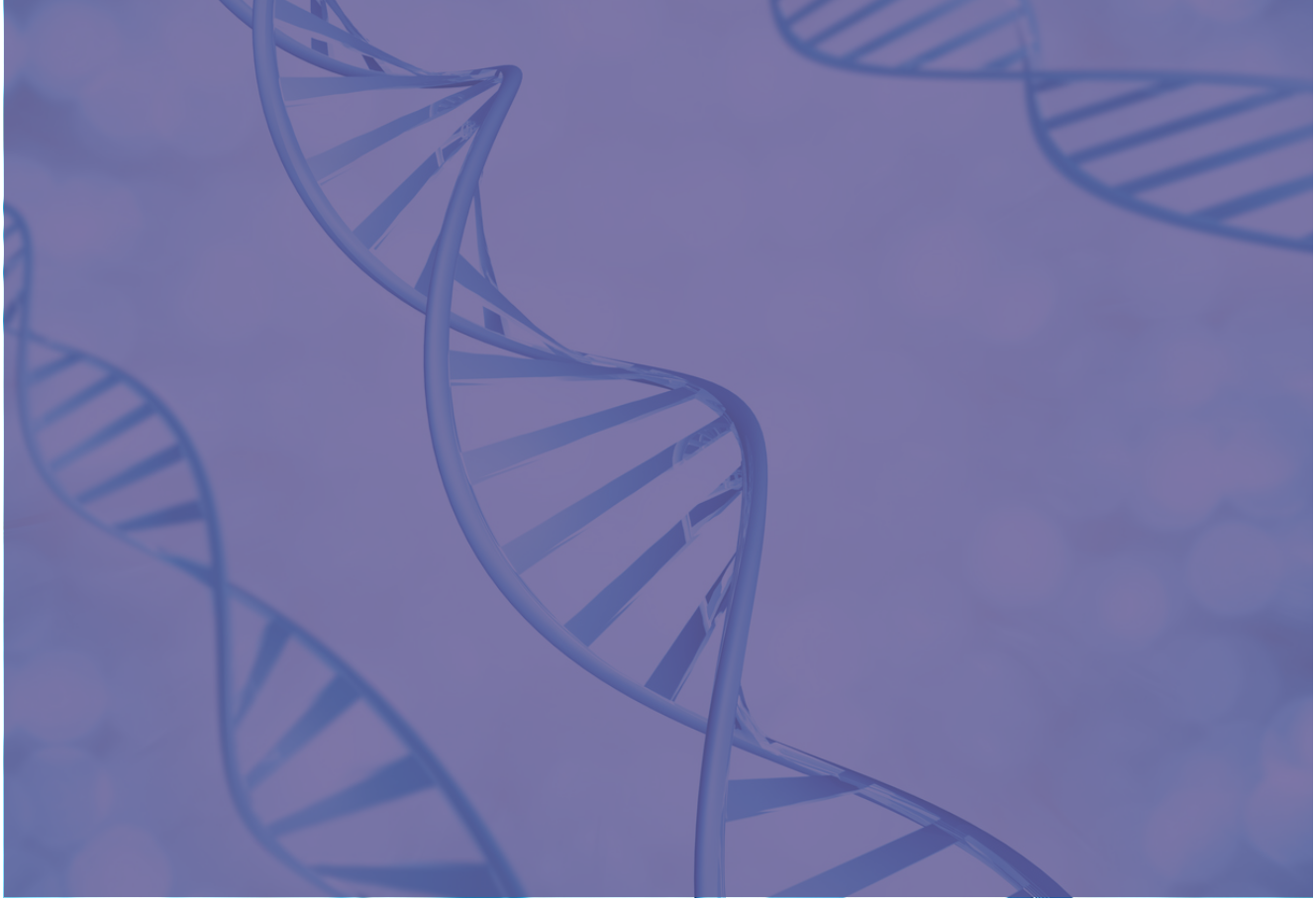
Matsuura, S., Matsushita, Y., Usugi, T., Tsuda, S., 2010. Disinfection of tomato chlorotic dwarf viroid by chemical and biological agents. Crop Prot. 29, 1157 1161.

Roistacher, C.N., Calavan, E.C., Blue, R.L., 1969. Citrus exocortis virus – chemical inactivation on tools, tolerance to heat and separation of isolates. Plant Dis. Reptr. 53,333 336.

Singh, R.P., Boucher, A., Somerville, T.H., 1989. Evaluation of chemicals for disinfection of laboratory equipment exposed to potato spindle tuber viroid. Am. Potato J. 66,239 245.

Thi Thu, 2018. Studies on transmissibility, cytopathology and control of Tomato chlorotic dwarf viroid and Potato spindle tuber viroid. Doctoral Thesis, Bonn University

Timmermann, C., Mu¨ hlbach, H.P., Bandte, M., Bu¨ ttner, C., 2001. Control of mechanical viroid transmission by the disinfection of tables and tools. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol. Wet. 66, 151 156.



This comprehensive report reviews 10 papers with over 50 experiments conducted to determine the most effective methods of tool and equipment sterilization to prevent the transmission of Hop Latent Viroid (HLVd) in cannabis.

At TUMI Genomics we recommend consistent and periodic testing to effectively control for HLVd.

In upcoming publications, we will provide Standard Operating Procedures (SOPs) that will help professional growers to maintain a clean operation.

info@tumigenomics.com

320 E. Vine Dr, Suite 213 - Fort Collins, CO 80524

(720) 807-8864

www.tumigenomics.com