

ISSUES AND MITIGATIONS

Hop Latent Viroid

DR. JOHN BRUNSTEIN

CHIEF SCIENTIFIC OFFICER, SEGRA INTERNATIONAL

invested in growth

seggra



**A MESSAGE FROM JAMIE BLUNDELL
CHIEF EXECUTIVE OFFICER, SEGRA INTERNATIONAL**

Large-scale crop loss is every grower's nightmare, but unfortunately, it is becoming an increasingly common reality. Modern *Cannabis* pathogens such as Hop Latent Viroid (HLVd) are emerging and accelerating, particularly in California, the most mature cannabis market in the world. While California has long been an inspiration to the global cannabis community as an established and thriving market, it now also needs to serve as a cautionary tale as global cannabis operators come into contact with a growing list of pathogens causing documented economic losses.

HLVd is just one in a growing list of identified intercellular pathogens that will be identified in *Cannabis* in the years to come.

Legalization, with its move from small- to large-scale monoculture practices, provides a fertile ground for the emergence of such pests in the unending battle between hosts and pathogens. Plant tissue culture, active pathogen detection strategies, and best farming practices form a key trio of mitigation measures that must be adopted as industry best practices in order for growers to have a fighting chance against HLVd, and a host of other pathogenic invaders.

From our company's inception, we have invested strategically in the development of our quality assurance and pathogen detection systems to complement our nursery product offerings.

We have currently developed six internal assays for key viral, fungal and bacterial *Cannabis* pathogens, and in 2021 are investing additional resources towards the development of other key pathogen assays. All cultivars available for purchase from Segra have been tested negative for a range of pathogens, including HLVd. In addition to our in-house screening, we engage with a variety of independent external testing labs that provide independent validation of our work.

As part of our continuous effort to support the spread of best practices in the industry, we'd like to take this opportunity to share the steps Segra and many of our clients are taking to mitigate this HLVd risk. Dr. John Brunstein, our Chief Scientific Officer, has assembled this document outlining best practices for detecting, managing, and mitigating HLVd. As a PhD virologist, Dr. Brunstein speaks with a high level of qualification on this specific subject.

We hope you find this information valuable. Our team is ready to answer your questions and provide support. Please reach out if you need help.

Best,

Jamie Blundell
CEO, Segra International



invested in growth
Segra

Know What You Grow™



Hop Latent Viroid (HLVd)

From its prior history where many cannabis cultivators suggested *Cannabis sativa* "doesn't have viruses," the larger industry now is facing the reality that this isn't the case – and leading the push in this is probably Hop Latent Viroid (HLVd). HLVd is increasingly recognized as a widespread agent with an unknown but likely significant impact on large-scale cannabis crop yields.

HLVd infection, as seen on the right, attenuates flower and resin development. These plants of the same cultivar are the same age and grown under identical conditions.

Photography by: Ali Bektas

■ What is Hop Latent Viroid?

Well, it's a viroid – strictly, not the same as a virus. A virus has both a nucleic acid genome and a protein (and sometimes, also a lipid) shell to protect it and allow survival in the environment as it transmits between host plants. A viroid, by contrast, is purely naked genetic material. This makes it much less robust. Viroids in general don't transmit well between host plants (lateral transmission), relying mostly on spread within a single plant leading to the presence in seeds.

In the course of a normal plant life cycle, this leads to infected progeny (vertical transmission). Lateral transmission is still possible but generally only through direct mechanical means. For example, an insect feeding on an infected plant, or a tool used on an infected plant, cuts through the outer cell walls of an uninfected plant and moves an infectious inoculum of viroid into a new host.

HLVd is a ribonucleic acid (RNA) genome viroid, which further amplifies its weakness to environmental exposure as compared to a DNA-based pathogen. Understanding all of these points is critical in developing and assessing the utility of the various mitigation strategies that will be discussed below.



Stunted growth, as seen above, can be observed in plants where HLVd has taken hold. These plants of the same cultivar are the same age and grown under identical conditions.

■ How is it detected?

The first challenge in dealing with HLVD is in knowing when it is present. As the "latent" part of the name implies, it can exist in a host plant for a significant amount of time without overt signs of disease.

The method of choice for identifying HLVD is through detection of the viroid RNA's unique sequences through reverse transcription polymerase chain reaction (RT-PCR). This is ideally done in what's referred to as a "real-time" format.

■ How is Segra mitigating transmission?

At Segra, we have developed and validated an assay that takes into account all available HLVD sequence data and is now available in-house for QA purposes as of the beginning of March 2021.

This assay will be used to screen incoming material for Plant Tissue Culture (PTC) induction, and to track the "cleaning" of any infected incoming varieties. Repeat negative test results will be required before a variety will be brought into production. The test will also be used on an ongoing basis to confirm the "HLVD not detected" status of those cultivars in routine cultivation.

Rather than relying on older published HLVD detection assays, Segra's real-time RT-PCR assay is based on a consensus of current HLVD sequences recorded in Genbank. This primer set may detect additional sequence variants of HLVD that would otherwise score false negative with published methods based on older, less complete data.

Within Segra's PTC lab, risk of lateral transmission of HLVD (or similar pathogens) is extremely low. The sealed, separate-jars nature of PTC cultivation forms a physical barrier to pathogen transmission. Further, conducting all work within laminar flow hoods reduces the risk of aerosol particulate contamination routes and, importantly, in this context, the use of high-temperature bead sterilizers for all tools when moving between plants removes the opportunity for mechanical transfer - outlined above as the major risk for HLVD lateral transmission.

In short, presence of HLVD in one material is vanishingly low risk to other material/cultivars under the practices used.

Segra's Verified Clean Stock

At Segra, our Verified Clean Stock guarantee is not just an extra layer of protection, but the core of our business ethos.

In the face of increasingly urgent incursion of pathogens, terms like "clean stock" or "pathogen-free" are often used loosely and frequently misunderstood. They often describe programs that work to actively minimize pathogen risks using a variety of horticultural "elimination" or "isolation" strategies. But these pathogen-free claims are simply impossible on a nursery level, as our understanding of what lurks on a plant is limited to the pathogens we know and can test for, a list that continues to evolve. In the face of this, clean stock programs have emerged as a vital tool in protecting your grow.

But not all clean stock programs are created equal. For some nurseries, clean stock could mean a simple dip of clone cuttings in a light bleach solution. But the recent emergence of pathogenic virus/viroids in commercial cannabis grows places particular importance on tissue culture as a tenant of any clean stock program, as it is the only way to remove viruses from either clonal or seed-based propagation systems.

At Segra, our nursery operations are able to take clean stock further, creating programs that involve multiple tiers of pathogen screening, advanced tissue culture techniques, and long-term preventative planning.



■ How can cultivators mitigate transmission?

Receiving Segra's Verified Clean Stock plants has limited value to the cultivator if those plants get infected immediately on arrival at the grow facility.

As always, the best indicator of plant health is how it looks and how it is growing. Nothing can replace vigilance and quick response to possible infection, but some simple techniques can help to foster a healthier growing environment. With knowledge of the biology involved, it is possible to identify the two major risk factors for HLVD spread at a cultivator:

- Insect pests (thrips, aphids, etc.) which can act as vectors
- The use of improperly sterilized tools, such as pruning shears, when moving between plants

While high-temperature bead sterilization is the gold standard, it may not always be available or practical in a large grow situation; in these cases, use of a freshly (at least daily) prepared 10% domestic bleach solution and minimum 60-second immersion time for tools when moving between plants is a good option. This is highly effective at chemical disruption of any HLVD RNA, blocking its capacity to infect the next plant tissue contacted.

Use of alcohol sanitization, or bleach which is not freshly prepared, is ineffectual and leads to a false sense of security. Additionally, when working with mother stock plants, growers may choose to physically connect a single pair of pruning shears to each mother plant using rope or wire to prevent the use of that tool on any other plant.

In short, cultivators need to be:

- Starting any grow cycle with Verified Clean Stock plants
- Monitoring for and addressing insect pest issues
- Sterilizing all tools appropriately when moving between plants

When these steps are taken, most of the significant means for HLVD lateral transmission are addressed. As a bonus, these measures are also effective against many other potential pathogenic microorganisms, including ones not yet even identified. Use best practices!

Why Plant Tissue Culture?

Plant Tissue Culture (PTC) is a process used worldwide as a best practice for almost every clonally reproduced crop, including berries and orchids. In the coming years, it will be cemented as best practice for cannabis cultivation as well.

PTC technology is central to our Verified Clean Stock program as it allows for the return of cultivars to a revitalized, clean stock state where they are liberated from disease organisms associated with traditional vegetative propagation methods.

In addition to these benefits, PTC plantlets have also shown increased dry flower yield (up to 30%) and cannabinoid contents (up to 17%) compared to "clones" produced from traditional vegetative propagation methods.

Segra offers a full range of PTC services that help to identify and protect IP, regenerate aging and diseased cultivars, diversify your product offering, or replace your mother room altogether.

IN SUMMARY:

What you need to know about Hop Latent Viroid

1

HLVD is likely just the first of a list of identified intercellular pathogens that will be identified in *Cannabis* in the years to come.

2

Legalization, with its move from small- to large-scale monoculture practices, provides a fertile ground for the emergence of pests in the unending battle between hosts and pathogens.

3

The method of choice for detecting HLVD is through detection of the viroid RNA's unique sequences via reverse transcription polymerase chain reaction (RT-PCR). This is ideally done in what's referred to as a "real-time" format.

4

To minimize the risk of HLVD lateral transmission, cultivators need to:

- Start any grow cycle with Verified Clean Stock plants
- Monitor for and address insect pest issues
- Sterilize all tools appropriately when moving between plants



The best trio of mitigation measures against HLVD and other future pests:

Tissue Culture
Active Detection Strategies
Best Handling Practices

Segra is your best choice for healthy plant starts and is ready to partner with your organization to ensure you remain out in front of HLVD and future identified intercellular pathogens.

Get in touch today.

Carson Otto
Director of Sales
carson.otto@segra-intl.com
905-802-6533