Engineering RNA Interference Precursors to Induce Silencing of Hop Latent Viroid in Hop (*Humulus lupulus* (L).)

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Introduction:

Hop Latent Viroid (HLVd) is an economically impactful, infectious pathogen of hops (*Humulus Lupulus*) and cannabis (*Cannabis sativa*) which reduces yield and may alter the sensitive secondary metabolome of hop. Viroids are virus-like infections pathogens that are characterized by their short, non-coding, and highly structured RNA sequences lacking encapsulation. Ranging from 246-401 nucleotides (nt) long, these pathogens induce specific effects by means of hijacking host cell machinery. RNAi, a form of regulatory post-transcriptional gene silencing, is a means of host defense against fungi and viruses. Research into its success against viroids is essential as there are no current methods of viroid management in industries.

RNAi utilizes the RNA induced silencing complex (RISC) to induce silencing by slicing target mRNA. Using small RNA (sRNA) approximately 19-22nt long derived from the genome, RISC locates complimentary targets. Figure (1). reveals the relationship between viroid infection and host RNAi.

Hop is a vine-like perennial plant which produces cones containing lupulin glands responsible for secondary metabolite production (Fig. 2A). Secondary metabolites including alpha and beta bitter acids and essential oils, are extracted during beer production. Alteration of hop cone yield and secondary metabolites affect the taste and quantity of cones supplied to brewers. Similarly, infection in cannabis has conferred the nickname “dudding” disease.

HLVd is now detected in over thirteen countries where it causes incommensurate symptoms on the different species and cultivars of hop and cannabis.

The objective of this study is to develop an RNAi-based defense against HLVd utilizing dsRNA precursors (Fig. 2C).

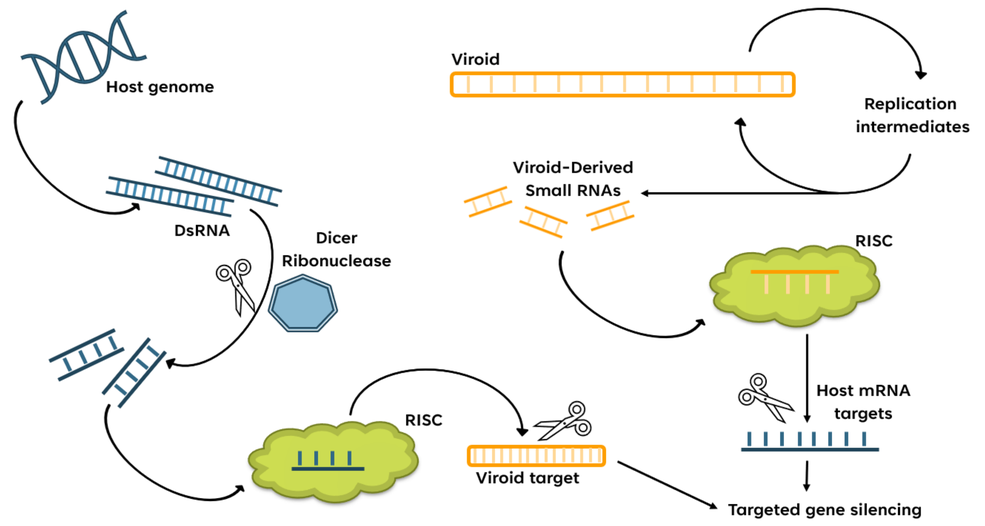
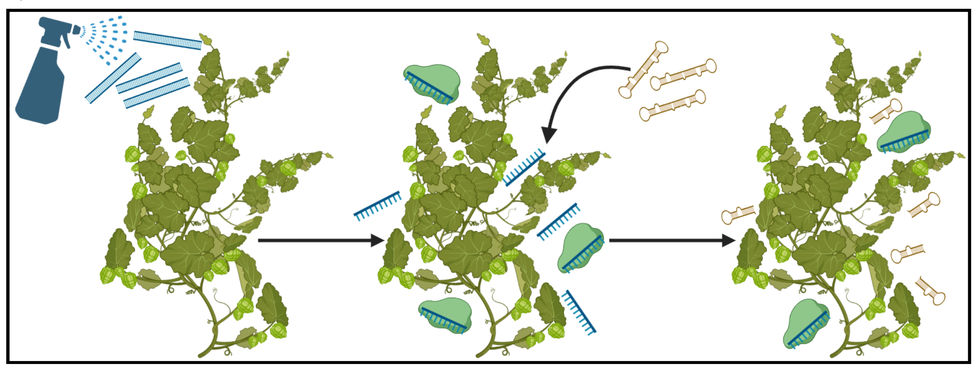


Figure 1: Simplified diagram of viroid interaction with host RNAi

A).

B).



C).

Figure 2: A). Image of hop during cone maturation. B). Image of hop yard where sampling occurred. C). Diagram of main objective of research

Objectives and Hypothesis:

The first objective of this study is conduct a survey on the available hop affiliated with the Ontario Crops Research Center - Simcoe, near Ontario (Fig. 1B) to determine frequency of HLVd presence and titer. Second is structural characterization of the HLVd genome to aid in determining an effective region to produce dsRNA precursors as guides for host RNAi (Fig. 3). Third, to determine most effective method of infecting healthy hop with the viroid. And finally, spray dsRNA precursors containing complementarity to decided viroid sequence on plants 24-48 hours prior to viroid infection and observe if preventative effects occur (Fig. 2C). It is hypothesized that application of RNAi precursors expressing high complementarity to essential regions of HLVd to hop (*Humulus lupulus* (L.)) plants prior to infection will reduce viroid manifestation.

Methods:

Twenty hop plants were randomly selected from a total of 272 available plants. 10 of each ‘Centennial’, and ‘Chinook ’ cultivar were selected (Fig 1B). The Norgen Biotek Plant/Fungi Total RNA Purification Kit was used for RNA extraction of all samples, including leaf, petiole, cone, and root tissues. Uniform sample sizes of 0.1 or 0.2g was used for all samples except cones due to structural fragility. Taqman RT-qPCR HLVd Norgen Biotek kit and protocol will be used to determine viroid presence and titer. Hop Latent Viroid (HLVd) is a member of the Posiviroidae family and shares a similar structural blueprint, including an unbranched conformation, the presence of a central conserved region (CCR), and the lack of a hammerhead ribozyme. Regions of the genome have been identified through comparison of recent research on HLVd and its most closely related family member (Fig. 3).

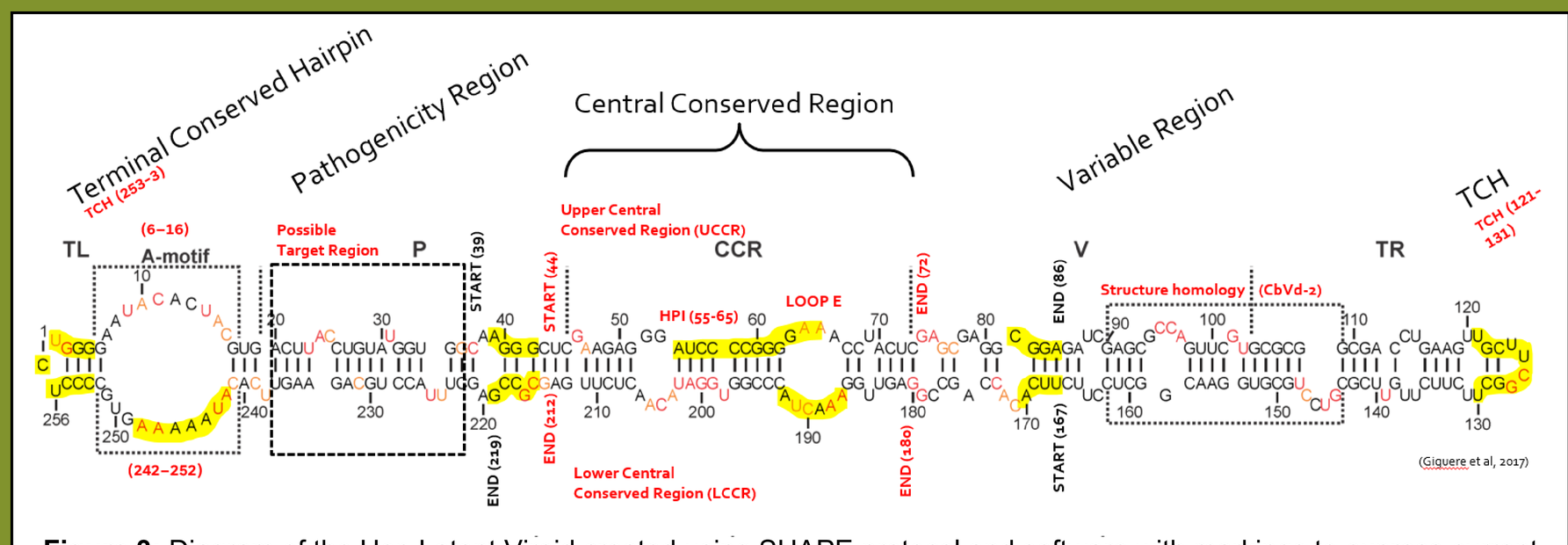


Figure 3: Diagram of the Hop Latent Viroid created using SHAPE protocol and software with markings to express current progress on on structural characterization

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