**COVER PAGE (2 pages, maximum)**

**Project Title: *Are the latent viruses really latent?***

**Project Summary:**

Of the six viruses actively managed by the U.S. hop industry, Hop latent virus and American hop latent virus, are ‘latent’ and not associated with clear disease symptoms. Existing viral disease management advice is to remove infected plants, even if no symptoms are presented by individual viruses. This is due a) to the risk of the pathogen(s) spreading into susceptible hosts, and b) the cumulative yield loss over time attributed to viral infection. But is this an appropriate use of resources and/or management practices?

This study aims to answer that question by determining whether the two latent viruses present a significant risk to the U.S. hop industry by examining their pathogenicity and effect on five economically important cultivars over a period of three years. The effect of these viruses alone and in combination with other, more pathogenic viruses will be assessed, with an emphasis on plant health and sustainability, and examining growth and physiology, virus accumulation, effect on photosynthesis, and product quality. From this data, best management practices will be drafted and disseminated to growers and producers in the U.S.

**Proposed Duration:** Continuing Proposal, Year 3 of 3.

**Project Leader:** Dr. Scott Harper

Washington State University

24106 N. Bunn Rd

Prosser, WA 33950

509-786-9230

scott.harper@wsu.edu

**Amount Requested:**

FY2022 (Year 3 of 3):

**Other Funding Sources and Support**

None

**Send Funding To:**

Katy Roberts, CAHNRS Grant Manager, College of Agricultural, Human and Natural Resource Sciences, Washington State University

**Project Title: *Are the latent viruses really latent?***

**Statement of Problem:**

There are six common viruses or virus-like organisms infecting Hops in the U.S., of which Hop latent virus (HLV) and American hop latent virus (AHLV) are ‘latent’ and are thus not known to cause specific disease symptoms, such as yellowing, mosaic, or decline, on hop cultivars. There are older reports of these two viruses causing yield losses on specific cultivars but given the state of virus diagnostics at the time of these reports were made, losses may not be attributed solely to these viruses.

These two viruses are ubiquitous in commercial hop cultivars throughout the country, due in part to their aphid transmissibility. Under current state certification rules and best management practices, these two viruses are considered potentially pathogenic and are actively managed, with growers testing for and removing infected plants.

The ubiquity of these viruses, and the potential of false negatives from older diagnostic assays which calls the validity of their results into question, raises the need for studies to determine the effects of these two latent viruses by themselves, in mixture together, and with other viruses, on hop productivity to answer the question of whether they should be managed, or whether the cost of plant removal and management is greater than the effect of these viruses. This work, therefore, addresses the priorities of sustainability, as well as field and yard management over the short and long term.

**Justification and Importance of Proposed Research:**

In the past two decades little work has been performed on the effects of either Hop latent virus, or American hop latent virus. HLV has been reported to cause chlorotic flecking on Hersbrucker Spat, while AHLV is asymptomatic (Pethybridge et al. 2008). Both viruses have been reported to cause yield losses, characterized as shorter nodes and laterals, and fewer leaves, of between 13-15% (Probasco & Murphey 1996). Yet recent developments in diagnostics techniques, particularly the application of real-time RT-qPCR, have revealed that that these two viruses are ubiquitous in U.S. hops, with incidences of 60% and 53% in virus positive samples tested in 2018 alone. Furthermore, HLV has been found in apples in Washington state, suggesting that growers must not only be concerned with hop-to-hop transmission, but transmission from neighboring crops as well (Harper, unpublished).

Yet these two viruses are actively managed through diagnostic testing and removal of infected plants, resulting in lost productivity as well as the need for replanting. These are costs that are currently being absorbed by the industry, with little to no scientific justification. Therefore we propose to examine the pathogenicity of these two viruses on common commercial hop cultivars, and establish whether these two viruses need to be managed, or whether they industry can safely live with their presence, and focus on viruses and other pathogens that do cause significant damage to hops.

**Objective(s):**

1. Determine the pathogenicity of *Hop latent virus* and *American hop latent virus* in common commercial hop cultivars over multiple growing seasons both by themselves and in mixture with other common, pathogenic hop viruses.
2. Recommend an appropriate management strategy *Hop latent virus* and *American hop latent virus* for US hop growers.

**Procedures/Methods to accomplish objectives:**

Objective 1: A series of 6 commercial cultivars (Citra, Cascade, Zeus, Centennial, Chinook and Triumph) for which virus-free lines exist at the CPCNW will be selected, propagated in greenhouse facilities at the WSU-IAREC site and inoculated with *Hop latent virus*, *American hop latent virus*, or both viruses together by inoculation using aphids in replicates of 5. Additional replicates will be added with these two viruses, singly or together, in the presence of either *Apple mosaic virus*, or *Hop mosaic virus* to represent the potential effects of coinfection with a pathogenic virus. Once inoculated, plants will be tested by RT-qPCR for viral presence before proceeding.

Plants will be scaled up to 5-gal root training pots and primary shoots trained up wires to approximate field production conditions over the next three years, being pruned back to ground level each winter and allowed to re-grow. First, observations will be made for the expression of viral-disease like symptoms, such as chlorosis or stunting. Measurements will be taken of plant height, shoot and node length, average leaf mass, and when available, cone mass, on a monthly basis and compared by analysis of variance. Measurements of phytosynthetic potential will be made on a monthly basis, and chlorophyll fluorescence examined as chlorosis or yellowing is a common disease symptom that can be difficult to compare and/or diagnose visually. The effect on alpha and beta acids will be assessed by HPLC from a sample of dried cones in the second and third years. Finally, virus accumulation and titer will be examined on a quarterly basis by real-time RT-qPCR using extant assays developed in this lab, to determine whether there are synergistic interactions occurring between the targeted viruses.

This study will run for a period of three years, or specifically, three growing seasons, as losses to viral infection are often cumulative, with disease expression taking 1-2 years to fully express in a new planting, and also, disease can be inconsistent between years, therefore it is important to perform the study over a time period sufficient to capture variation.

Objective 2: This is contingent on the outcome of Objective 1 and is to formulate a management strategy based on the findings of the pathogenicity study. Recommendations will be made for different scenarios, such as Hop latent virus alone, and in the presence of other viruses.

**Outcomes**

Objectives 1 and 2: Short term, the data gathered in this project will help develop a management strategy for these two common viruses; in the mid and long term, growers will be able to use these recommendations to make informed decisions about how to use diagnostic testing and whether to remove or simply monitor plants for disease, apply spray programs, scout for disease expression, or change management practices.

**Extension and Outreach Activities**:

Management recommendations from Objective 2 will be prepared as a factsheet, as well as an academic publication, and distributed to growers via USAHops and university extension agents. The researchers will also present findings at state meetings in Washington, Oregon, and Idaho.

**Time Frame for Objectives**

*Due to 1) funds for the first year being received in April 2020, delaying the commencement of the project, and 2) the Covid-19 outbreak in 2020, which severely impacted on-site laboratory research and plant propagation, the timeframes for activities have been shifted from the original proposal into years 2 and 3.*

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| --- | --- | --- |
| **Year 1 (2020-2021)** | **Year 2 (2021-2022)** | **Year 3 (2022-2023)** |
| 1st Qtr | 2nd Qtr | 3rd Qtr | 4th Qtr | 1st Qtr | 2nd Qtr | 3rd Qtr | 4th Qtr | 1st Qtr | 2nd Qtr | 3rd Qtr | 4th Qtr |
|   | Propagate and inoculate selected cultivars |   |   |   |   |   |   |   |   |
|   |   |   |  | Assess viral load and population structure |   |
|   |   |   |   | Examine pathogenesis and physiological characteristics on a monthly basis. |   |
|   |   |   |   |   |   | Assess effect on cone yield and content |   |   | Assess effect on cone yield and content |
|   |   |   |   |   |   |   |   |   |   | Assess risk and prepare recommendations on management practices |

**Year 2, progress update**

1. To ensure that the presence of other non-pathogenic viruses and viroids could influence the results of this study, lines of ‘Cascade’, ‘Zeus’, ‘Centennial’, ‘Chinook’ and ‘Triumph’ held at the Clean Plant Center Northwest were regenerated from excised meristems, removing Hop latent viroid (which is ubiquitous in U.S. hops and whose presence is accepted as part of the hop certification program). These plants have been propagated and transferred to soil in anticipating of inoculation and planting.
2. The commercial line ‘Citra’ has been obtained and is undergoing cleanup as the source was infected with latent viruses. Meristem regeneration has been successful and the plantlets are undergoing rooting prior to transfer and confirmation of their virus-free status before the experiment can commence.
3. A colony of hop aphids (*Phorodon humuli*) has been established and is undergoing sequential transfer with each generation of nymphs to ensure no virus is present prior to use.
4. Inoculum sources have been obtained, propagated, and tested to ensure that only the targeted viruses are present, and at titer sufficient to successfully perform aphid inoculation.

**Project Budget**

|  |  |  |  |
| --- | --- | --- | --- |
| **Year 3 (FY 2022)** | **Hop Research Council Request** | **Commission Request** | **Total Amount Requested** |
| **State:** WA |
| WHC |
| Salaries | 18,303 | 18,303 | 36,606 |
| Employee benefits | 1,536 | 1,536 | 3,072 |
| Temporary or hourly workers | 0 | 0 | 0 |
| Travel (to Hop Conv.) | 0 | 0 | 0 |
| USA Hop Convention Registration | 0 | 0 | 0 |
| Equipment  | 0 | 0 | 0 |
| Other: Lab reagents and consumables | 5000 | 5000 | 10,000 |
| **Total** | 24,839 | 24,839 | 49,678 |

**Other Funding Sources and Support**

SCRI (PI: D. Walsh) “Enhancing Supply Chain Sustainability and Global Competitiveness for Pacific Northwest Hops” commencing 9/1/21.

**Literature Review**

There are six common virus or virus-like organisms infecting Hops in the US: Apple mosaic virus, Hop mosaic virus, Hop stunt viroid, Hop latent viroid, Hop latent virus, and American hop latent virus. Of these, the first three have been demonstrated to be pathogenic and cause disease on commercial hops (Pethybridge et al. 2008). The last three all have ‘latent’ in their names, a viral nomenclature term that indicates no disease was associated when the organisms were first found and identified (Probasco and Skotland 1978). Of these, Hop latent viroid is near-ubiquitous in commercial hop lines (Pethybridge et al. 2008), and while not reported to have significant pathogenic effects itself, it has been implicated in causing disease when co-infected with Citrus bark cracking viroid (Jakse et al. 2015); fortunately, Citrus bark cracking viroid, while present in the US in citrus groves in California and Florida, is not found near hop production areas and has a minimal risk of introduction (Harper, unpublished).

This leaves us with two latent viruses, Hop latent virus and American Hop latent virus, both members of the Carlavirus genus, and somewhat similar genetically (Hataya et al. 2000), whose effects on Hop cultivars are poorly described, if at all (Adams and Barbara 1982). Hop latent virus has been reported to cause chlorotic flecking on Cluster (Probasco and Skotland 1978) and Hersbrucker Spat (Pethybridge et al. 2008), but not on cultivars from the Golding or Wye lineages (Adams and Barbara 1982), nor on most of the common extant commercial cultivars held in the CPCNW collection (Harper, unpublished). No diagnosable symptoms have been reported for American hop latent virus on any cultivars (Pethybridge et al. 2008).

Both viruses have been reported to cause yield losses, characterized as shorter nodes and laterals, and fewer leaves, of between 13-15% (Probasco & Murphey 1996), and Hop latent virus has demonstrated with small decreases in alpha and beta acid content by itself, but may synergistically increase loss when in combination with more pathogenic viruses (Pethybridge et al. 2002). Yet, the advances in diagnostic assays and technology have led researchers and growers to question these results, for it has been found that they are far more ubiquitous and prevalent in commercial hop lines that previously suspected, and were present in 60% and 53% of virus positive samples tested in 2018 (Harper, unpublished). For comparison, incidence of Hop latent virus in Australia is as high as 90% (Pethybridge et al. 2000). Furthermore, real-time RT-qPCR assays suggest that many of these infections are at low titer, and were hence undetectable by older techniques such as ELISA or endpoint RT-PCR.

Furthermore, Hop latent virus is being actively transmitted in the field by *Macrosiphum euphorbiae,* the potato aphid, and *Myzus persicae*, the green peach aphid, a species that is prolific and highly mobile (Crowle et al. 2006). Both Hop latent virus and American hop latent virus are also transmitted by *Phorodon humuli*, the hop aphid (Adams and Barbara 1982). Hop latent virus has been found to infect not only hops, but weeds and ornamental species such as Dianthus (Adams & Barbara 1982), and more recently, Apples (Harper, unpublished). It is therefore unlikely that a long-term planting in the U.S. will remain uninfected for long, raising the question of what management strategies are appropriate.

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