**Assessing seasonal host use and pathogen transmission by beet leafhopper**

**in vegetable and hemp crop systems**

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**I. Project Summary**

Pathogens transmitted by insect vectors such as beet leafhopper (*Circulifer tenellus*) Baker (Homoptera: Cicadellidae) affect many agricultural crops and wild hosts. In the Western US, one such pathogen is beet curly top virus (BCTV; family Geminiviridae, genus Curtovirus), which can cause considerable losses in crops such as beet, carrot, tomato, hemp, radish, pepper, and vegetable seeds. Control methods for BCTV primarily involve the management of leafhopper vectors. However, leafhoppers are notoriously difficult to control because they move among crop and non-crop hosts throughout seasons, and it remains unknown which weedy hosts are sources of infectious leafhoppers. To address these knowledge gaps, my project was designed to achieve four objectives: (1) link pest monitoring and molecular tools to assess prevalence of leafhopper-transmitted pathogens across hosts, (2) assess feeding habits of leafhoppers found in potato crops using gut content analyses, (3) assess whether leafhopper host use is consistent across growing regions, and (4) investigate whether BCTV evolves to specific hosts. To achieve objectives 1 and 2, my prior work involved sampling beet leafhoppers and associated host plants across Columbia Basin potato fields from 2019 to 2022. Plant tissue from sampling events was tested for leafhopper-transmitted pathogens, and leafhoppers were subjected to molecular gut content analysis to assess feeding patterns. For objective 3, in 2022 I expanded my leafhopper sampling to non-potato crops, and in 2024 and 2025 I plan to expand this work further and work with collaborators in Oregon and Colorado. Results will indicate which hosts are widely used by leafhoppers during seasons and will allow us to infer pathways of transmission among weeds and crops. My fourth objective assesses how the BCTV pathogen evolves in different host species, and whether pathogen transmission among hosts changes over time. Overall, my research will provide considerable information on the natural history and ecology of leafhoppers and associated pathogens and will aid in developing management tactics for these pathogens. More broadly, my work shows how molecular techniques can complement traditional monitoring efforts to better understand the dynamics of insect vectors and vector-borne plant pathogens.

**II.** **Project Narrative**

***A. Justification and Long-Term Goal of the Project***

Most viral crop pathogens are carried and transmitted by insects. Despite the importance of vector-borne plant viruses for agriculture, our ability to predict their occurrence across time and space remains poor. The long-term goal of my dissertation project is to leverage molecular tools along with ecological studies to investigate the dynamics of a key vector of vegetable crops in the Pacific Northwest USA, the beet leafhopper. My project will first use molecular tools and field monitoring studies to assess leafhopper host use and pathogen transmission across growing seasons. My field studies will then be complemented with laboratory studies that assess how specific viral pathogens transmitted by leafhoppers evolve to specific hosts, and how pathogen transmissibility among hosts is maintained over time. My dissertation project involves four specific chapters, each of which will contribute to our understanding of leafhoppers and plant pathogens. More broadly, my work will demonstrate how high-throughput molecular tools can complement traditional pest monitoring to aid in building decision-making tools for pests.

***B. Background and Literature Review***

*(i) Specialty crop production in the Pacific Northwest US*

High-value specialty crops like potatoes, tomatoes, beets, carrots, and coriander are key to the agricultural economy of Washington state and the Pacific Northwest more broadly. Notably, Washington boasts the highest yields of potatoes in the US, with over 20% of US production occurring in the Columbia River Basin region. Alternative crops like hemp are also being widely introduced to the region and are grown in close proximity to other vegetable and seed crops. As crops such as potato can only be grown once every 3 to 4 years (due to high nutrient demand), growers are dependent on viable production of multiple crops for economic sustainability.

*(ii) Threats to specialty crop production in the Pacific Northwest US*

One of the primary factors hindering production of specialty crops like potato and hemp is disease, and many pathogens affecting crops are transmitted by insect vectors. For example, the beet leafhopper (*Circulifer tenellus*) feeds on numerous crops and transmits several devastating pathogens like BCTV and *Candidatus* Phytoplasma trifolii (CPt) (Cooper et al 2023). Beet leafhoppers have a gut adaptation that allows phloem pathogens to traverse their stomach lining and colonize their salivary glands, making them efficient vectors (Knowlton 1929, Suzuki et al 2006). Beet leafhopper-transmitted pathogens threaten crops including potato (*Solanum tuberosum),* tomato (*Solanum lycopersicum*),various seed crops (*Raphanus sativus*, *Daucus carota*, *Phaseolus spp.*, *Brassica spp.*), peppers (*Capsicum spp.*), hemp (*Cannabis sativa*), and sugar beets (*Beta vulgaris*) (Hudson et al 2010; Nachappa et al 2020; Rondon and Murphy 2016; Soto and Gilbertson 2003). In particular, a strong resurgence of BCTV in the Western US has occurred since 2019, with disease incidence in non-potato crops reaching as high as 90% plants infected (Nachappa et al 2020; Swisher Grimm et al 2021). The continuous annual return of these pathogens is attributed to the life cycle of their vectors, where overwintering leafhoppers emerge in the spring and acquire pathogens from weedy hosts before moving into crops. ***Despite the constant movement of leafhoppers between weedy and crop hosts, we still have a relatively poor understanding of leafhopper ecology that can be used to predict pathogen outbreaks***.

*(iii) Leafhopper ecology makes management difficult*

Beet leafhoppers are highly polyphagous and move among crop and non-crop hosts within seasons. Beet leafhoppers undergo three generations each year in the Pacific Northwest US, with adults and larvae initially favoring weeds in families Amaranthaceae and Brassicaceae as hosts (Hudson et al. 2010). As temperatures rise in mid-April and weeds dry out, leafhoppers migrate to summer crops, spreading pathogens. At the end of summer, females carrying fertilized eggs seek shelter, flying in search of newly germinated winter weedy perennials or annuals before they diapause. The considerable host diversity across landscapes makes it so beet leafhoppers have an abundance of suitable hosts throughout the environment to support populations.

Well-suited for the polyphagous lifestyle of beet leafhoppers, pathogens such as BCTV are transmitted in a persistent manner to an extensive range of wild and crop hosts across more than 300 species (Harveson 2015). BCTV has a ssDNA genome and has at least 11 strains that beet leafhoppers can transmit (Harveson 2015, Melgarejo et al 2022). Adult leafhoppers can carry BCTV for months and inoculate new hosts within a minute of feeding (Frantz et al 2023). Prior research indicates that leafhoppers do not distinguish between plant types during initial feeding bouts and will feed on plants that are not suitable for reproduction (Thomas and Boll 1977). All of these factors contribute to leafhoppers spreading pathogens efficiently across hosts, and ***understanding interactions between pathogens, vectors, and hosts is key for developing effective pest management strategies that can reduce economic losses and enhance crop yields.***

*(iv) Leafhoppers may exhibit unique host use patterns across regions*

The introduction of hemp, tomato, and other susceptible specialty crops into the Columbia Basin region may be impacting leafhopper populations and incidence of pathogens like BCTV. As climates warm, the range of leafhoppers is expanding, and management of this group of insect vectors may benefit from a broader understanding of host use and pathogen transmission in other crop production regions. In particular, Oregon and Colorado have similar crop diversity to Washington State, and coordination between groups studying beet leafhoppers in these states will improve understanding of impacts of plant availability, regional pathogen strain information, and BCTV epidemiology. ***In this proposal we will work collaboratively with several research groups to better understand how the introduction or increase in acreage of more susceptible crops like tomato and hemp may be impacting the recent rise in incidence of BCTV.***

*(v) Virus evolution and molecular mechanisms employed by BCTV*

The genome of BCTV encodes coat proteins and several open-reading frames involved in viral replication, gene expression, and host manipulation (Lozano-Duran et al 2011, Luna et al 2020). Infection leads to stunting and leaf curl symptoms in hosts by manipulating host plant physiology and evading the plant's defenses, mainly impacting plant metabolism, particularly the metabolism and transport of carbohydrates, leading to changes in levels of sugars like fructose and glucose (Majumdar et al 2022; Villa-Ruano et al 2018).

Given the need to manipulate hosts for transmission, characterization of BCTV strains has shown considerable strain diversity; in particular, plant samples associated with outbreaks has revealed the emergence of new recombinant strains. Recombinant isolates have been found with 2.2 kb length of their genome sequences homologous to the Worland strain but carrying a 770-nucleotide fragment homologous to the CFH sequence, which partially spans the coding frames for the C1 and C2 genes (Rondon et al 2016). C1 proteins are involved in BCTV replication and C2 proteins affect symptom expression. The magnitude of symptom expression is dependent on the virus C2 interaction with plant accessions’ defense and transcriptomic responses (Son et al 2014). These interactions affect the plant's response to stress hormones and cell cycle regulation. BCTV interferes with the ubiquitination machinery of the host cell by inhibiting CUL1-based SCF ubiquitin E3 ligase and inducing the activity of RING finger protein RKP, a functional ubiquitin E3 ligase (Lai et al 2009). These interactions lead to abnormal cell division and affect viral replication. Geminiviruses, like BCTV, target host DNA methylation and RNA silencing processes to evade host defenses (Jackel et al 2015). BCTV uses proteins like MET1 and ADK to manipulate DNA methylation and suppress RNA silencing (Guo et al 2018).

Overall, BCTV employs a complex array of molecular strategies to manipulate host plant physiology, evade host defenses, and promote its own replication and spread. For these reasons, the virus may evolve to specific host plant species. We predict that if we force BCTV into a single species for long enough that mutations will occur in the C1 and/or C2 region to specialize effect on host cells. If true, over time BCTV will lose its ability to be transmitted across host species but will increase its ability to be transmitted within plant populations of a single host. ***Understanding how BCTV evolves to specific hosts will aid in understanding how the virus adapts in new environments and how host diversity affects virus spread.***

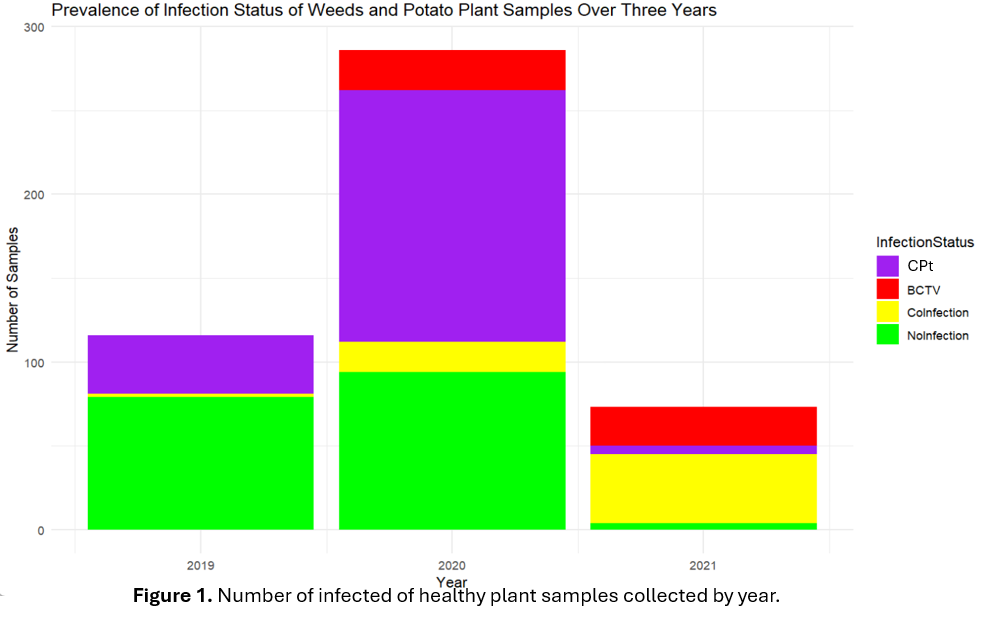
*(vi) Literature review summary*

Specialty crop production is essential to the economy of the Pacific Northwest, as growers produce a variety of vegetable, seed, and fiber crops. One of the most persistent threats to crop production in this region is insect-borne pathogens such as BCTV, which affect many hosts. Polyphagous vectors such as beet leafhopper, which transmits BCTV, are notoriously difficult to manage because they move between crop and non-crop hosts; management of the vector in any single crop is thus not sufficient to achieve an areawide reduction. To improve management of leafhoppers, we need a better understanding of how leafhoppers use hosts throughout growing seasons. Identifying which hosts function as key sources of leafhoppers and pathogens may aid in predicting movement into crop fields. More broadly, understanding how pathogens can be transmitted among hosts over seasons and evolutionary time would aid in predicting routes of transmission. In this proposal we will link molecular techniques with field and laboratory studies to better understand leafhopper-borne pathogens and their effects on a variety of host species.

***C. Preliminary Data***

*(i) Assessing incidence of two pathogens in leafhoppers and hosts*

To assess the distribution of leafhoppers, hosts, and pathogens, we collected plant samples from potato fields and nearby weeds from 2019 to 2021, with the number of sites varying from 4 to 15 each year. Fig. 1 shows the infection status for plants collected per year. In 2019, 114 total plants were collected with an even distribution of 20 kochia, 20 pigweed, 20 potato, 20 Russian thistle, and the rest mustards and amaranths. Of these 114, 35 samples were positive for CPt and 3 were positive for BCTV, of which all three were co-infected with CPt and BCTV. In 2020, more samples were collected with more variety, at a total of 250 plants collected, consisting of 60 Russian thistle, 51 potato, 40 kochia, 22 netseed lambsquarter, 14 pigweed, and mustards. Out of these 250 plants, 150 were positive for CPt and 24 positive for BCTV, of which 18 were co-infected. The sampling size was smaller in 2021, with 73 plants collected with 25 flixweed, 22 blue mustard, 14 tumble mustard, and 9 kochia. Of these 73 plants, 46 were positive for CPt and 64 were positive for BCTV with 41 of those samples showing co-infection. This confirms local weed species as sources of pathogens and highlight the quick increase in BCTV prevalence.



Data for infected plant collections from all three years were pooled together to create a total for each species infected. Results of any species sampled less than ten times was removed for analyses. Weeds commonly found in the landscape like various brushes, pigweed, and prickly lettuce had low infection rates. Fig. 2 shows abundance of infected plants represented by circle size with larger circles having more samples. Each species total of both infected and healthy plants was used to create a proportion for how many of each plant species was infected with either pathogen. A main host of beet leafhoppers is Russian thistle but Russian thistle shows only half samples infected with BCTV and less than 25% infected with CPt. The next most abundant species is kochia, showing half of samples infected with BCTV and over 25% infected with CPt. Collections were focused around potato making it the next most abundant species. Potato had almost 0% infection BCTV, confirming previous reports of tolerance in potatoes to BCTV, and over 75% infection with CPt, a prominent disease for this crop type. Other species of interest include various Brassicaceae like flixweed, and blue or black mustards.

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In 2022, we expanded our sampling to include symptomatic hemp samples from WSU’s Othello Research farm. Of 38 samples collected, 37 were positive for BCTV. To determine the strain, some samples were sent to Colorado State University for sequencing, resulting in the identification of two mild types, BCTV-CO and BCTV-Wor. Of the remaining samples, 24 were tested for CPt with only 2 testing positive, both of which were co-infected.

*(ii) Gut content analysis*

To begin to assess host use by leafhoppers, molecular gut content analysis was performed on individual beet leafhopperscollected from 2019 to 2020. Beet leafhoppers found on yellow sticky cardswere removed and surface sterilized before DNA extraction, followed by pathogen identification by quantitative real time polymerase chain reactions (qRT-PCR). Each individual beet leafhopper was then barcoded with a unique ITS2 and trnF primer pair before being sequenced with Pacifica Biosciences (PacBio) Single-molecule, real-time sequencing. Our results show beet leafhopper weedy hosts are mainly in the families Amaranthaceae, Asteraceae, Brassicaceae, and Solanaceae. Most prevalent Amaranthaceae are Russian thistle (*Salsola tragus*, S. *komarovii, Kali turgidum*), kochia (*Bassia scoparia*), and pigweed (*Amaranthus hybridus*). Most Asteraceae include prickly lettuce (*Lactuca serriola*) and fleabane (*Erigeron strigosus, E. canadensis*). The Brassicaceae included mustards (*Brassica spp.*, *Sysymbrium altissimum*) and hoary cress (*Cardaria draba*), with Solanaceae comprising mostly nightshades (*Solanum spp.*). These results are consistent with previous visual reports (Munyaneza et al. 2008, 2010; Hudson et al 2010). Fig. 3 shows the most prevalent genera total found from individual beet leafhopper guts. The most prominent genus is Solanum, followed by Russian thistle (Kali and Salsola). In addition to weedy hosts, the main crop hosts of beet leafhopper were potato (*Solanum tuberosum*), sugar beet (*Beta vulgaris*), tomato (*Solanum lycopersicum*), beans (Phaseolus spp.), peppers (Capsicum spp.), various vegetable seed (*Raphanus sativus, Daucus carota*),and other crops (Brassica spp*., Cannabis sativa*) (Hudson et al 2010; Munyaneza 2005; Nachappa et al 2020; Rondon and Murphy 2016; Soto and Gilbertson 2003). Gut content analysis also showed considerable amounts of high value crops species such as radish (*Raphanus sativus)*, lettuce (*Lactuca sativa*), and rapeseed (*Brassica carinata*).

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**Figure 3 –** Number of genera found in individual beet leafhoppers from 2019-2021

Data did not show significant difference in infection status between host species type, but what did stand out was the number of species per infected leafhopper gut. Insects infected with BCTV had less plants in guts than insects not infected or infected with CPt. This could possibly be due to the nature of each pathogen’s lifecycle. BCTV can only replicated within plant hosts and is non-replicative within the leafhopper. Infection of BCTV alters insect behavior and causes them to move less, essentially so that they may feed more on BCTV infected hosts and increase their uptake of virus loads. Fig. 4 shows a decline in BCTV infection as more plants are found within leafhopper gut. The more plants eaten the less virus load, as healthy plants dilute BCTV within the insect. In contrast, CPt does proliferate within leafhopper hosts, which can eventually lead to death of the insect by becoming over filled with the bacteria. Since the leafhopper is producing the pathogen, it behooves the bacteria lifecycle for the vector to move more to spread to more hosts. Infection of CPt remains somewhat constant based on number of plants per belly.

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*(iii) Summary of preliminary data*

Our preliminary data confirms which regional plants are most often infected with BCTV, CPt, or both and most likely green bridges for these pathogens to crops. The plant samples also indicate seasonality of pathogen threat, with mustards and flixweeds being more available and infected in early spring, kochia becoming infected early summer and maintaining infection until fall, and Russian thistle surprisingly having low infection. To confirm that these plants are hosts of beet leafhoppers, gut content analysis highlights the most prominent genera that are fed upon. These plants found in the gut are then correlated to the infection status of the beet leafhopper, indicating which plants are most likely green bridges and fed upon most by infected vectors. It is not surprising that Brassicaceae are favored for overwintering and through early spring as these plants grow cold-tolerant rosettes. In 2019, CPt was consistently found throughout the season and the three BCTV were all found in July. In 2020 and 2021, infection of CPt was found throughout the season with BCTV infection being strongest in early summer/June and tapering down near the end of the growing season. This is also expected as the winter hosts die off, the virus concentration is diluted as leafhoppers disperse throughout less susceptible fields. We also know that plants can outgrow BCTV so younger plants will have higher titers of BCTV. This data highlights the most important sources of pathogens throughout the Columbia Basin and gives temporal data for predicting when crop risk is greatest.

***D. Objectives***

This research proposal aims to leverage various molecular techniques with field and lab studies to better understand the dynamics of beet leafhopper-transmitted pathogens. This project encompasses four complementary objectives:

(i) Objective 1: Link pest monitoring and molecular tools to assess prevalence of leafhopper-transmitted pathogens across hosts

(ii) Objective 2: Assess feeding habits of leafhoppers in potato crops using gut content analyses

(iii) Objective 3: Assess whether leafhopper host use is consistent across crops and regions

(iv) Objective 4: Investigate whether BCTV evolves to specific hosts

***E. Approach***

*(i) Objective 1:* *Link pest monitoring and molecular tools to assess prevalence of leafhopper-transmitted pathogens across hosts*

(a) Rationale and Hypothesis*.*One of the primary objectives of this project is to identify the seasonal hosts that are sources of infectious leafhoppers to crops, and those hosts that support the overwintering of leafhoppers and pathogens. We predict that leafhopper host use, and incidence of BCTV and CPt, will vary based on geographic region and seasonality. More specifically, we predict that 1(a) mustards and flixweed will have highest association with BCTV prior to crop emergence, 1(b) kochia will have high association with BCTV throughout the growing season, and 1(c) Russian thistle will have low infection of BCTV. A better understanding of the hosts that leafhoppers use throughout the season, and which have the highest incidence of pathogens, would aid in predicting risk to crops and developing proactive management tactics.

(b) Methods*.* As mentioned earlier, leafhopper and plant samples around potato fields were collected in the Columbia Basin from 2019 to 2022 and tested for the presence of CPt and BCTV using PCR. One new growth leaf per plant was sampled, beginning as early as March, or when hosts were free of snow, until October. Individual plant samples were stored at –80°C until ground in mortar pestles with liquid nitrogen to a fine powder. The plant powder was used to extract DNA using a DNeasy Plant Mini Isolation kit (Qiagen, Inc., Valencia, CA) following standard protocols, except incubation at 65°C for 20 min instead of 5 min, and use of 50 µL of Buffer AE in the final step instead of 100 µL for stronger DNA concentrations.

The DNA was first nanodropped for quality control and then PCRs were performed using a CFX thermocycler (Bio-Rad). For BCTV identification, coat protein primers that amplify a 496-bp fragment from curtovirus-related species were used: BCTV2-F 5′-GTGGATCAATTTCCAG-ACAATTATC-3′ and BCTV2-R 5′-CCCATAAGAGCCATATCAAACTTC-3′ (Strausbaugh et al 2008). Reactions were performed in 96-well plates with volumes of 10 μL with components in the master mix at following concentrations: 1× PCR buffer (5uL SYBRgreen, 3uL PCR-grade water), 1uL Fwd and Rvs primers mixed at 20uM, and 1 uL DNA. Amplification consisted of 5 minutes at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 54°C, and 1 min at 72°C. After the last cycle, 5 min at 72°C ran before the reaction was held at 4°C (Strausbaugh et al 2008).

Samples were tested for CPt using *Candidatus* Phytoplasma trifolii specific primers z-R16R2-wfB-F: AAATATTTCTCGGGGTTTGTACACACCGCCCGTCA and BLTVA-int-wfB-R: AATTATCTCTGATGATTTTAGTATATATAGTCC. Polymerase chain reactions (PCR) were performed on a CFX thermocycler (Bio-Rad) or Lightcycler 480 (Roche) in volumes of 20 µ with the components per well at the following amounts: 8.2ul PCR grade water (included in Lightcycler 480 SYBRgreen kit), 0.4ul (20uM) z-R16R2-wfb, 0.4ul (20uM) BLTVA-int-wfb, 10ul 2x Lightcycler 480 SYBRgreen I Master (Roche Diagnostics), and finally 1ul DNA template. Thermocycler conditions for rt-PCR consisted of a pre-incubation period of 5:00 minutes at for 95oC, followed by a touchdown series (Δ-0.5°C) with 20 cycles of amplification containing 10 seconds at 95oC, 10 seconds 65oC, followed by 10 seconds 72oC. After the touchdown cycles, another 20 cycles at 10 seconds at 95oC, 10 seconds 55oC, followed by 10 seconds 72oC. Amplification cycles were followed by a melt curve at 95oC for 5sec (4.4C/s ramp rate), 65oC for 1min (2.2C/s ramp rate), and then the melt curve target of 97oC (0.11C/s ramp rate) with 5 acquisitions per second. After the melt curve regime there is a final cooling step of 40oC for 10sec. (Cooper et al 2023, Swisher-Grimm et al 2023).

(c) Data analysis and expected results*.*We will use logistic regression models to assess whether the prevalence of BCTV and CPt are associated with host species that beet leafhoppers were collected from. We expect that if certain hosts are common sources of pathogens, that there will be positive correlations between incidence of these hosts in leafhopper guts and pathogen incidence. We expect the seasonality to vary as hosts change in abundance. We predict that the seasonality of beet leafhoppers will be consistent enough to determine that mustards and flixweed will have highest association with BCTV in early spring, infection by BCTV of kochia will grow throughout the season, and that Russian thistle, although a main host of leafhoppers, will have low infection of BCTV throughout the summer. These results confirm that local hosts are sources of pathogens and highlight which hosts are major players in the transmission of pathogens from year to year. We did not expect to find such low infection in Russian thistle. More research is needed to understand the lack of pathogen prevalence of this host type.

(d) Potential pitfalls and contingencies*.* Pitfalls to this experiment are the bias of collection locations and the lack of recording the diversity abundance of plant species per location. This experiment can provide a generalized survey for the Columbia Basin. In general, the most abundant weeds in the region are Russian thistle and kochia which could influence data by indicating an absence of evidence in other species rather than evidence of absence. Another pitfall could be the optimization of DNA extraction per species type. All plants followed the same protocol, yet each plant has different defenses, metabolites, and especially for these species types, trichomes present, which could impact the quality of extraction. Russian thistle extractions were of a dark purple to black color, possibly due to higher contents of anthocyanins or other water soluble flavonoids. These plant compounds could be interacting with molecular outcomes or could be influencing insect behavior, either leading to lower BCTV results.

*(ii) Objective 2: Assess feeding habits of leafhoppers in potato crops using gut content analyses*

(a) Rationale and hypothesis. The second objective involves performing gut content analysis on beet leafhoppers collected from potato fields and various weed species throughout the Columbia Basin to identify host plants fed upon. This will provide valuable insights into the host use of leafhoppers infected with BCTV and CPt. We have the following predictions: 2(a) beet leafhoppersinfected with BCTV in early summer will have more mustards and flixweed in their diet, 2(b) the presence of kochia in the insect gut will be associated with higher prevalence of co-infection of both CPt and BCTV, 2(c) leafhoppers infected with BCTV will have greater diversity of Solanaceae in their gut, and 2(d) insects infected with BCTV will have less genera in gut. This objective will reveal seasonal preferences and when these plant hosts are most prevalent, which can aid in the development of targeted weed and pest management plans.

(b) Methods. To identify which plants are most fed upon by beet leafhoppers infected with BCTV, CPt, or both, host identification was tested on samples from 2019 to 2021 using qRT-PCR and barcode primers. Beet leafhopper populations were monitored and collected bi-weekly using recommended yellow sticky cards (Crosslin et al 2012; Munyaneza et al 2008; Munyaneza et al 2010), as well as sweep netting and DVAC leaf blower collecting. Yellow sticky traps were placed around the edges of potato or crop fields and in nearby weedy areas at ground height or vegetation height, as rate of capture decreases as trap height increases (Meyerdirk and Oldfield 1985). Sampling began as early as March and went until October. Individual leafhopperswere surface sterilized with rinses of bleach, ethanol, and deionized water to remove contaminants from the sticky traps. Qiagen DNeasy Blood and Tissue kits were used to extract DNA from individual beet leafhoppers*.* Each individual sample of beet leafhopper DNA were barcoded, or indexed, using two unique primer sets for both ITS2 and trnF assigned via PCR.

PCR were performed in volumes of 50 µl with the components per well at the following amounts: 25 µL Amplitaq Gold 360 MasterMix, 15 µL PCR-grade water, 2.5 µL assigned Fwd primer at working stock 5µM, 2.5 µL assigned Rvs primer at working stock 5µM, and 5 µL DNA (either water for NTC, positive control of known feeding extracted either psyllid or beet leafhopper DNA to ensure the primer pairs worked, or DNA for each sample). The entire batch sent for sequencing also contained a negative control of just water which should not show up in sequence reads and a positive control of known feeding extracted either psyllid or beet leafhopper DNA which should only show either tomato or beet, respectively.

Thermocycler conditions for PCR varied by primer type and optimization. For all ITS pre-incubation period consisted of 5 minutes at 94°C, followed by 35 cycles of amplification containing 30 seconds at 94°C, 30 seconds 56°C, followed by 45 seconds 72°C. After the amplification cycles, a final 5 minutes at 72°C was followed by a 4°C hold. For trnf PCRs, most samples were run at thermocycler conditions of pre-incubation period consisting of 10 minutes at 94°C was followed by 40 cycles of amplification containing 30 seconds at 94°C, 30 seconds 58°C, followed by 45 seconds 72°C. After the amplification cycles, a final 5 minutes at 72°C was followed by a 4°C hold. For a few plates, the trnF primers were optimized for the annealing temperature to be at 52°C instead of 58°C for the following protocol of; pre-incubation period consisting of 10 minutes at 94°C was followed by 40 cycles of amplification containing 30 seconds at 94°C, 30 seconds 52°C, followed by 45 seconds 72°C. After the amplification cycles, a final 5 minutes at 72°C was followed by holding at 4°C (Cooper et al 2016).

Quantity and quality of DNA were checked using Nanodrop with any 260/280 or 230/280 ratios below 1.5 removed. Quantity and quality of barcoding was checked by running an electrophoresis gel for band brightness. PCR product was cleaned using a Qiagen PCR Clean-Up Kit. Once individual DNA was barcoded and cleaned, all samples were pooled together and sequenced by WSU’s Genomics lab using PacBio. Fasta data from PacBio sequencing was input and cleaned on Geneious Prime software platform by Dotmatics, an industry-leading bioinformatics and molecular biology tool for streamlining sequence analysis. Data was exported from Geneious Prime to Excel for further cleaning and combined with beet leafhopper collection and pathogen data, then uploaded in Rstudio for analysis.

Gut content analysis of beet leafhopper collected from the Columbia Basin 2019-2021 was performed to create projections for timing of seasonal hosts correlating with BCTV and CPt. In 2019, 64 individual beet leafhoppers were sequenced for gut content analysis. To get a more comprehensive picture of which hosts are most beneficial to leafhoppers, individuals in 2019 were selected based on the plant type they were collected from with even distribution between kochia, potato, russian thistle, and pigweed, with a few random mustards. In 2020, individuals were chosen based on infection status with 97 beet leafhopper sequenced with 24 BCTV, 25 CPt, 23 Co-infected BCTV+CPt, and 25 healthy individuals. In 2021, infection status for BCTV was not tested until after gut content analysis was performed, with 73 individual beet leafhopper chosen based on seasonality with stronger focus on early spring collections and CPt status.

(c) Data analysis and expected results. We will use logistic regression to assess whether presence of hosts in the gut are associated with BCTV or CPt. We predict that infection will influence the diet of leafhoppers, and that leafhoppers infected with BCTV have more diversity of Solanaceae in their guts. We also found that BCTV-infected leafhoppers have less genera in their gut which is expected as researchers at Colorado State University are finding BCTV to affect leafhopper muscles, making them move less (unpublished Nachappa). We found high amounts of potato in all leafhoppers as collection was focused around potato fields. What stands out is that BCTV infected leafhoppers also had potato, yet potato are tolerant to BCTV. Further research is needed to better understand the mechanisms that make potato tolerant to BCTV.

(d) Potential pitfalls and contingencies. Pitfalls to this experiment include the degradation of DNA while the insects are on the yellow sticky traps. To aid in validating results, we will not only look at the presence of hosts but also the number of sequences. However, the number of sequences can be confusing as perhaps the leafhopper had fed on that plant long ago indicating lower sequence numbers, or possibly they only fed on that plant for a little amount. The primers used for barcoding also have specificity towards certain genera. We used two primer types per sample to help capture the most data but ITS are more generic while trnF are specialized to weeds leading to different results. As primer specificity and DNA degradation are expected, we plan to only validate results to the genus level as species may be misleading misreads.

*(iii) Objective 3: Assess whether leafhopper host use is consistent across crops and regions*

(a) Rationale and hypothesis. The third objective is an expansion of objective 2 to have a more comprehensive understanding of the range and effects of available host plants on infection of beet leafhoppers by performing gut content analysis on leafhoppers collected from a variety of specialty crops throughout several states. Beet leafhopper DNA from insects from vegetable seed and hemp crops in Washington, Oregon, and Colorado will be sequenced to determine seasonal plant hosts. Data will be used to help assess the phenology of winter weed hosts and the activity of beet leafhoppers, allowing for a more accurate risk assessment of associated vectored diseases. By understanding the timing of leafhopper migration and disease transmission, we can optimize the timing of pest management interventions.Such collaboration is essential, as pests like beet leafhoppers’ boundaries expand beyond the state and this data will aid in understanding population migrations. Gut content data can be used for developing predictive pest management models in combination with precipitation and temperature patterns following WSU’s Decision Aid System, an online platform for growers to monitor insect life stages and pathogen levels. This objective will promote education, extension outreach events, and multi-state collaborations.

(b) Methods. To identify which plants are most fed upon by beet leafhoppers collected from specialty crops throughout Washington, Oregon, and Colorado, gut content analysis will be performed following Objective 2, but the focus will shift from potato crops to regional specialty crops like hemp and vegetable seed crops. Samples from Washington will be collected using yellow sticky traps and extracted using Qiagen kits, as described above. Researchers from each state will send leafhopper DNA to be barcoded and sequenced to determine host plants of local vectors.Outreach and surveys will be conducted to expand the knowledge of WSU’s Decision Aid System (DAS) from the Columbia Basin to be used as a baseline for other states to enhance their pest management systems. Information and brochures about DAS will be disseminated at several conferences to benefit society and agriculture. A larger network of researchers and educators will foster collaboration between growing regions affected by localized populations of vector beet leafhopper to improve information on agricultural epidemiology and diversity of pathogenic strains across the Western United States. This objective will address how regional availability of hosts influences infection of BCTV in beet leafhoppers and how host availability may affect different BCTV strain(s) abundance. We expect that, Hypothesis 3(a) Beet leafhoppers collected from specialty crops will have less potato in gut content than leafhoppers caught from potato fields. Hypothesis 3(b) Beet leafhoppers from Colorado will have less diverse gut content than beet leafhoppers from the Columbia Basin. Hypothesis 3(c) Beet leafhoppers from Colorado will have more diverse BCTV strains than beet leafhoppers from the Columbia Basin.

(c) Data analysis and expected results*.* Our data analysis will follow Objective 2, although we will look at correlations on a region by region basis, and then qualitatively compare whether host use and patterns of infection differ across regions. We predict that availability of hosts will influence of abundance of pathogens. When more susceptible species are available, we predict that more pathogens will be present. We also predict to see less potatoes in gut contents and more specialty crops. Each region being tested contain high amounts of kochia and Russian thistle which is assumed to be consistent main hosts, but we predict to see differences in the next most prominent hosts as variability in the landscape will influence variability in gut contents.

(d) Potential pitfalls and contingencies. Pitfalls to this experiment are the same as Objective 2 with the addition of differences in techniques for DNA extraction leading to differences in ability to sequence gut contents. Another pitfall might be that the landscapes of the regions are too similar for differences to be observed between states. With out surveying the landscapes it will be impossible to determine if leafhopper gut contents is due to abundance or preference. Another confounding factor may be that these beet leafhopper populations are actually one larger population.

*(iv) Objective 4: Investigate whether BCTV evolves to specific hosts*

(a) Rationale and hypothesis. Plant viruses frequently evolve in response to host genomes for a more targeted attack. Surveys of BCTV have identified varying prevalence of unique of isolates in specific regions with particular crops being grown, suggesting dynamic changes in the strain composition of BCTV found in leafhopper vectors (Strausbaugh et al 2008). BCTV strains also often exist co-infected as a complex, which can be differentiated by genetic sequences and biological properties (Gilbertson). Previous research has identified mild curly top (BCTV-CO and -LH71) predominantly in tomatoes, other crops, and weeds, except for severe curly top (BCTV-Srv CFH) in sugar beets, showing a partiality of strains for certain crops (Gilbertson). Enhancing our comprehension of virus evolution will enable more effective management of existing diseases and the prevention of future outbreaks and enrich our understanding of the dynamics of evolutionary processes. To grasp the evolution of viruses comprehensively, it's essential to examine the various factors that influence their evolutionary trajectory, and this objective will test 3 hypotheses: Hypothesis 4(a) host type affects BCTV transmission. Hypothesis 4(b) BCTV will evolve to specialize within species type. Hypothesis 4(c) evolved BCTV will transmit better to same species of specialization.

(b) Methods. To study BCTV evolution, greenhouse experiments will force BCTV into one cultivar type for a time series of five passages. This will be performed on three host species: tomato (*Solanum lycopersicum*), carrot (*Daucus carota*), and fiber hemp (*Cannabis sativa*). These plant types were chosen as tomato and hemp are both susceptible to BCTV and their acreage is increasing in the Columbia Basin. Carrot is mostly grown as a biennial seed crop in the area, and the overwintering rosettes provide places of refuge for beet leafhoppers.

The virus evolution experiment will be performed on plants direct seeded and placed into isolation cages in greenhouses maintained at 73-77°F during the day and 59-63°F at night with 16-h light/8-h dark photoperiod. The original infections will be initiated using beet leafhoppers infected with BCTV. These leafhoppers have colonized four cages specializing on sugar beets and the original virus will be sequenced as the baseline for comparisons in changes to the genome. One adult BCTV-infected leafhopper will randomly be collected from each of the four BCTV beet colony cages and placed into a mesh sleeve inserted over a new growth leaf of a single tomato plant (Plant 1) placed within an isolation cage for the next 48 hours to ensure feeding and transmission of BCTV (Munyaneza and Upton 2005). After the 48 hr period, the 4 leafhoppers will be removed for DNA extraction and qRT-PCR to titer BCTV viral load per insect. This will be repeated for both hemp and carrot species.

Upon inoculation, plants will be inspected daily for visual symptoms. The infectivity data during the 14 days post inoculation (dpi) will be used to calculate an area under disease progress curve (*AUDPC)*. This formula transforms data from disease progression, allowing us to express the virulence and dynamics of the disease into a single figure. The *AUDPC* ranges between zero and the total number of observation time points along the experiment; larger values mean that the virus infects plants more quickly. *AUDPC* values can be computed using the agricolae R package with R in RStudio. and data can be fitted to two fully factorial generalized linear model.

A week after Passage 1, or 7 dpi of Plant 1, four clean adult beet leafhoppers from each of the four clean beet colonies will be removed, and placed onto the leaf of Plant 1 closest to the leaf of infection for 48 hrs. After 48 hrs the leafhoppers will be transferred to Plant 2 for 48 hrs to infect the new plant. The leaf tissue of the Plant 1 will then be removed, immediately frozen, and titered for BCTV levels. This inoculation process will continue for three more passages to allow the virus to specialize to host type. Fig. 5 shows an illustration of the experimental design, starting on the left with the original virus from beet colonies being moved into a specific species type. The virus will then be transferred from the plant of infection to the next host until we reach the fifth passage. Plant samples will be collected and sequenced from passage 1 and passage 5 for a total of 96 samples to be sent for Sanger sequencing for analysis of viral nucleotide and protein sequences. Plant DNA will be extracted using DNeasy Plant Pro Kit (Qiagen), and samples will be sequenced using Sanger libraries, Illumina sequencing (paired end, 150 bp), and quality check of the DNA-seq libraries were done by Novogene Europe. For viral genome SNP calling, trimmed reads will be mapped with HiSat2 to the original BCTV isolate focusin C1 or C2 with a modified minimum score parameter.

A diagram of a plant

Description automatically generated

**Figure 5**: illustration of experimental design for BCTV evolution

We will also determine if order of hosts impacts BCTV spread. For example, recent studies show that beet transmission to tomato is common but tomato transmission to beet is unlikely (Frantz et al 2023). Although tomatoes may be susceptible hosts, they may be poor sources of pathogen. As gut content analysis has provided which species are being fed upon, we will decipher how the order of feeding affects the spread of virus. To test transmission ability, the prior experiment will be replicated but expanded to include cross over for species type. On the first and fifth passage, species type will be crossed to both other species to compare transmission. The experiment will be replicated four times per species for a total of 72 leaf samples to be titered from treatment 1 and for treatment 5. Disease progress will also be calculated using *AUDPC.* This will determine if interspecific specialization of BCTV effect transmission between species types. Fig. 6 shows an illustration of the experimental design, starting on the left with the original virus from beet colonies being moved into a specific species. The arrows branching out off of the first and fourth treatment indicate species cross over. The plants the arrows point to are the samples that will be recorded and collected for viral loads.

A diagram of a plant

Description automatically generated with medium confidence

**Figure 6**: Illustration of experimental design for BCTV transmission

(c) Data analysis and expected results. We predict availability of hosts will influence evolution of BCTV to host specialization. If forced into a single species type, we predict BCTV will change genomic sequences in the C1/C2 region. We predict that after the time series of being forced into a single species type, transmission of BCTV will have greater success to a plant of the same species. We also predict that certain cannabis chemovars will have more susceptibility to BCTV than other chemovars, and the virus will evolve to specific chemovars.

(d) Potential pitfalls and contingencies. Pitfalls to this experiment can be maintaining virus-free beet leafhopper colonies. Also, the virus may not evolve during the duration of the experiment time. If this is the case, the experiment will be repeated but with the virus from treatment 5 to continue another 5 treatments reaching 10 successions. Another pitfall might be in the trust of virus titers as the virus moves unevenly throughout the plant and may not be represented evenly. To maintain consistency, leafhoppers will be placed on the newest growth leaf in a mesh bag and only that leaf will be used for titering.

***F. Expected Outputs and Outcomes***

Combining phenological data of beet leafhopperand susceptible available cultivars will aid determining migration patterns and in risk assessments used for spray calendar regimes. Larger impacts apply to other plant-pathogen-vector relationships and improving precision of integrated pest management. We present multiple lines of evidence, including PCR pathogen identification, gut content analysis, and greenhouse approaches, to demonstrate that changes in beet leafhopper feeding mediates pathogenic communities. These results suggest that metabolic profiles and microbial communities may play an unappreciated role in increasing plant transmission to pests by decreasing plant resistance (Blundell et al 2020).Accomplishment of these aims will provide insight into the population structure of beet leafhoppers. Divergent gut content and availability of host may point towards expression of pathogens, particularly if significant divergence is found between regional pathogen titers and seasonal environmental impacts. The long-term goals are to be used for improving precision of timing of pesticide applications. As visual diagnosis has been unreliable and created a need for molecular diagnosis, more economical, time sensitive, and immediate predictive methods are needed for future management methods of vectors.

***G. Intellectual Merits***

As globalization continues to expand trade, it becomes increasingly essential to consider the growing accessibility of insects and the diseases they carry in regions previously unaffected. Changing habitats now offer opportunities for invasive pest species to thrive. For instance, the establishment of the Columbia Basin Irrigation Project has transformed the climate of central Washington from a desert into a flourishing crop landscape. This ecological shift has affected the phenology of the beet leafhopper considerably (Cook 1967; Hills 1937, Horton et al 2018).

Enhancing our understanding of the beet leafhopper as a vector for diseases can lead to more precise integrated pest management methods. This precision can help reduce the usage of pesticides while simultaneously increasing agricultural yields. Recent research and theoretical models suggest a connection between disease prevalence and the seasonal availability of overwintering weeds, which is influenced by weather conditions. However, the role of host virulence in this relationship remains unknown. Given the increasing demand for higher crop yields, it is crucial to expand our knowledge of how pathogens can alter host plants, thereby influencing their attractiveness to vector insects. This study can serve as a model for accurate comparative molecular and ecological analyses in other vector species. Such insights can elucidate how the environment shapes virulence and its correlation with vector behavior.

This research aims to improve our comprehension of the intricate interplay between plants, pathogens, and vectors. It will shed light on how pathogens affect a plant host's relationship with its vector. Ultimately, this work is expected to advance our understanding of the broader implications of the plant-pathogen-vector dynamic on agricultural systems.

***H. Broader Impacts***

My research will contribute significantly to society by providing valuable data for sharing with growers through WSU's Decision Aid System, which is used to enhance pest management methods. Additionally, it will establish a broad network of researchers and educators, facilitating the dissemination of information and fostering collaboration to enhance our understanding of factors influencing vector behavior, agricultural epidemiology, and the diversity of pathogenic strains associated with the vector beet leafhopper across the Western United States.

A more comprehensive understanding of how annual weather patterns affect the virulence and fecundity of vectors will enable the development of more effective tools for integrated pest management. This knowledge will assist in determining optimal conditions for pathogens, thereby aiding in risk predictions. By expanding collaboration among Western states, we aim to create a more holistic picture of the epidemiology of diseases such as BCTV and CPt as they spread across the agricultural landscape in the Western region.

Our goals are aligned with the promotion of timely and cost-effective management of insect pests in specialty crops. This involves the integration of models and decision support tools to increase crop yields and quality while simultaneously reducing disease incidence. Achieving these objectives relies on improving the timing of pest management applications and enhancing the precision of pest control methods, ultimately reducing agricultural inputs.

***I. Impacts and Future Direction***

Understanding the disease cycle of BCTV and similar pathogens, coupled with insights into insect behavior and plant pathology, has the potential to revolutionize pest management strategies. Decreasing pesticide use while increasing crop yields remains a key goal. This research contributes to the improvement of predictions through WSU's Decision Aid System. The adoption of these monitoring systems in Washington has led to a significant reduction in insecticide use, resulting in substantial cost savings for growers.

In conclusion, this research not only sheds light on the dynamics of disease transmission by the beet leafhopper but also underscores the importance of collaborative efforts across states to combat the challenges posed by these insect vectors. Expanding these monitoring systems to other states may also reduce insecticide use and disease spread. Ultimately, the knowledge gained from this research has the potential to benefit growers and stakeholders across the region, ensuring the sustainability of specialty crop production.

**References**

Ball, E.D. 1907. The genus *Eutettix* with special reference to *E. tenella*, the beet leafhopper: a taxonomic, biologic and economic study of the North American forms. *Proc. Davenport Acad. Sci.* 12: 27–94.Blundell, R., Schmidt, J.E., Igwe, A. *et al.* 2020. Organic management promotes natural pest control through altered plant resistance to insects. *Nat. Plants* **6**, 483–491 (2020). <https://doi.org/10.1038/s41477-020-0656-9>.

Cook, W. C. 1967. Life history, host plants, and migrations of the beet leafhopper in the western United States. U.S.D.A. Tech. Bull. 1365. 122 pp.

Cooper W. R., Horton D. R., Unruh T. R., and Garczynski S. F. 2016. Gut Content Analysis of a Phloem-Feeding Insect, Bactericera cockerelli (Hemiptera: Triozidae). Environ Entomol. 2016 Aug;45(4):938-44. doi: 10.1093/ee/nvw060. Epub 2016 Jun 6. PMID: 27271944.

Cooper, W. R., Walker III, W. B., Angelella, G. M., Swisher Grimm, K. D., Foutz, J. J., Harper, S. J., Nottingham, L. B., Northfield, T. B., Wohleb C. H., & Strausbaugh, C. A. 2023. Bacterial endosymbionts identified from leafhopper (Hemiptera: Cicadellidae) vectors of phytoplasmas. *Environmental Entomology*, *52*(2), 243-253.

Crosslin, J. M., Rondon, S. I., & Hamm, P. B. 2012. Population dynamics of the beet leafhopper in northeastern Oregon and incidence of the beet leafhopper-transmitted virescence agent phytoplasma. *American journal of potato research*, *89*, 82-88.

Douglass, J. R., and W. C. Cook. 1952. The beet leafhopper. USDA Yearbook of Agriculture 1952: 544–550.

Frantz, R., Nischwitz, C., Compton, T., and L.F. Gordillo. Modeling the Spread of Curly Top Disease in Tomatoes. 2023. *Letters in Biomathematics*, *10*(1), 53-61. <https://doi.org/10.30707/LiB10.1.1682014077.8265>

Gilbertson, R. L., Beet curly top virus and other viruses of concern. <https://ucanr.edu/sites/Vegetable_Crops/files/227742.pdf>. PowerPoint Presentation

Guo, Z., Wang X.-B., Wang Y., Li W.-X., Gal-On, A. Ding S.-W. (2018) Identification of a new host factor required for antiviral RNAi and amplication of viral siRNAs. Plant Physiology, 175, 1587-1597.

Harries, F. H., & Douglass, J. R. 1948. Bionomic studies on the beet leafhopper. *Ecological Monographs*, *18*(1), 45-79.

Harveson, R. M. 2015. Beet curly top: America’s first serious disease of sugar beets. *Online publication. APS Features. doi*, *10*.

Hills, O. A. 1937. The beet leafhopper in the central Columbia river breeding area. *Journal of Agricultural Research*, *55*, 21.

Horton, D. R., Cooper, W. R., Swisher-Grimm, K. D., Crowder, D. W., Fu, Z., Waters, T. D., ... & Blua, M. (2018). The beet leafhopper odyssey in North America: a brief overview. *Potato Prog*, *18*, 1-10.

Hudson, A., Richman, D. B., Escobar, I., & Creamer, R. 2010. Comparison of the feeding behavior and genetics of beet leafhopper, Circulifer tenellus, populations from California and New Mexico. *Southwestern Entomologist*, *35*(3), 241-250.

Jackel JN, Buchmann RC, Singhal U, Bisaro DM. 2015. Analysis of geminivirus AL2 and L2 proteins reveals a novel AL2 silencing suppressor activity. J Virol. 2015 Mar;89(6):3176-87. doi: 10.1128/JVI.02625-14. Epub 2014 Dec 31. PMID: 25552721; PMCID: PMC4337558.

Knowlton, G. F. 1929. Bulletin No. 212-Studies on the Morphology of the Beet Leafhopper.Lai et al 2009

Lee, H., Wintermantel, W. M., Trumble, J. T., Fowles, T. M., & Nansen, C. 2022. Modeling and validation of oviposition by a polyphagous insect pest as a function of temperature and host plant species. *PloS one*, *17*(9), e0274003.Lozano-Duran et al 2011

Luna AP, Romero-Rodriguez B, Rosas-Diaz T, Cererol L, Rodriguez-Negrete EA, Castillo AG, Bejarano ER. 2020. Characterization of Curtovirus V2 Protein, a Functional Homolog of Begomovirus V2. Frontiers in plant science. 11:835–835. doi:10.3389/fpls.2020.00835.

Majumdar R, Galewski PJ, Eujayl I, Minocha R, Vincill E, Strausbaugh CA. 2022. Regulatory Roles of Small Non-coding RNAs in Sugar Beet Resistance Against *Beet curly top virus*. Front Plant Sci. 2022 Jan 10;12:780877. doi: 10.3389/fpls.2021.780877. PMID: 35082811; PMCID: PMC8786109.

Melgarejo TA, Chen L-F, Rojas MR, Schilder A, Gilbertson RL. 2022. Curly Top Disease of Hemp ( Cannabis sativa ) in California Is Caused by Mild-Type Strains of Beet curly top virus Often in Mixed Infection. Plant disease. 106(12):3022–3026. doi:10.1094/PDIS-04-22-0856-SC.

Munyaneza, J. E. 2005. Purple top disease and beet leafhopper-transmitted virescence agent (BLTVA) phytoplasma in potatoes of the Pacific Northwest of the United States. *Potato in progress: Science meets practice*, 211-220

Munyaneza, J. E., Crosslin, J. M., & Upton, J. E. 2006. Beet leafhopper (Hemiptera: Cicadellidae) transmits the Columbia Basin potato purple top phytoplasma to potatoes, beets, and weeds. *Journal of Economic Entomology*, *99*(2), 268-272.

Munyaneza, J. E., Crosslin, J. M., Upton, J. E., & Buchman, J. L. 2010. Incidence of the beet leafhopper-transmitted virescence agent phytoplasma in local populations of the beet leaf hopper, Circulifer tenellus, in Washington State. *Journal of Insect Science*, *10*(1), 18.

Munyaneza, J. E., Jensen, A. S., Hamm, P. B., & Upton, J. E. 2008. Seasonal occurrence and abundance of beet leafhopper in the potato growing region of Washington and Oregon Columbia Basin and Yakima Valley. *American Journal of Potato Research*, *85*, 77-84.

Munyaneza, J. E., & Upton, J. E. 2005. Beet leafhopper (Hemiptera: Cicadellidae) settling behavior, survival, and reproduction on selected host plants. *Journal of Economic Entomology*, *98*(6), 1824-1830

Nachappa, P., Fulladolsa, A. C., & Stenglein, M. 2020. Wild wild west: emerging viruses and viroids of hemp. *Outlooks on Pest Management*, *31*(4), 175-179.

Rondon, S. I., & Murphy, A. F. (2016). Monitoring and controlling the beet leafhopper Circulifer tenellus in the Columbia Basin. *American journal of potato research*, *93*, 80-85.

Rondon, S. I., Roster, M. S., Hamlin, L. L., Green, K. J., Karasev, A. V., & Crosslin, J. M. (2016). Characterization of beet curly top virus strains circulating in beet leafhoppers (Hemiptera: Cicadellidae) in Northeastern Oregon. *Plant Disease*, *100*(8), 1586-1590.

Son, S., Oh, C. J., & An, C. S. 2014. Arabidopsis thaliana remorins interact with SnRK1 and play a role in susceptibility to beet curly top virus and beet severe curly top virus. *The plant pathology journal*, *30*(3), 269.

Soto, M. J., & Gilbertson, R. L. 2003. Distribution and rate of movement of the curtovirus Beet mild curly top virus (family Geminiviridae) in the beet leafhopper. *Phytopathology*, *93*(4), 478-484.

Strausbaugh, C. A., Wintermantel, W. M., Gillen, A. M., & Eujayl, I. A. 2008. Curly top survey in the western United States. *Phytopathology*, *98*(11), 1212-1217.Suzuki et al 2006

Swisher Grimm, K.D., Crosslin, J.C., Cooper, W.R., Frost, K.E., du Toit, L.J., Wohleb, C.H. 2021. First report of curly top of Coriandrum sativum L. caused by beet curly top virus in the Columbia Basin of Washington State. Plant Disease. 105(10):3313. <https://doi.org/10.1094/PDIS-01-21-0041-PDN>.

Thomas, P. E., & Boll, R. K. 1977. Effect of host preference on transmission of curly top virus to tomato by the beet leafhopper. *Phytopathology*, *67*(7), 903-905.

Villa-Ruano, N., Velásquez-Valle, R., Zepeda-Vallejo, L. G., Pérez-Hernández, N., Velázquez-Ponce, M., Arcos-Adame, V. M., & Becerra-Martínez, E. 2018. 1H NMR-based metabolomic profiling for identification of metabolites in Capsicum annuum cv. mirasol infected by beet mild curly top virus (BMCTV). *Food Research International*, *106*, 870-877.