2024 WERA1007 - Curly Top virus Biology, Transmission, Ecology, and Management

Annual Meeting Dates: 06/17 – 06/18/2024

Report Date 08/10/2024

**Participants at Annual Meeting:**

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**Summary of Meeting Minutes**:

 **Rebecca Creamer**, WERA1007 Secretary, explained a bit about the group and its purpose. Introductions were made, and the agenda was discussed.

 **Bill Wintermantel** – USDA-ARS Salinas- welcomed the group to his new facility

**Rebecca Creamer** presented **Curly top overview and leafhopper life history in southern New Mexico**. She discussed the virus strains in found in NM in chile, sugarbeet, and hemp and host specificity of certain strains. The BLH appears to be univoltine since breeding cycles overlap. Some years midseason emergence of Kochia might contribute to midseason peaks in BLH. Late season peaks in BLH numbers and warm winters may lead to anomalously high numbers of BLH in the next season.

**Carl Strausbaugh,** USDA-ARS Kimberly, ID presented his work “**Beet leafhopper feeding and dispersal in Southern Idaho**”

Weeds and crop plants not only serve as reproductive hosts and transitory or shelter plants for the beet leafhopper (BLH; *Circulifer tenellus*) but also as sources of plant pathogens that can then be vectored by the BLH. Thus, the plants that the BLH are feeding on and infecting is of interest and may be changing over time. Therefore, BLH samples from a recent survey were investigated through DNA barcoding via the *rbcL* and *matK* chloroplast gene regions to determine what the BLHs had been feeding on prior to capture on yellow sticky cards in southern Idaho during 2020 and 2021. In June both years, the first generation of BLHs predominately fed on *Pinus* spp. (59 to 76% of samples) which were likely in mountainous areas and dispersed approximately 48 to 80 km to crop and sagebrush steppe locations. During July to September, the BLH predominantly fed on *Salsola* spp. (Russian thistle; 61 to 66% of samples) and *Bassia scoparia* (Kochia; 15% of samples). Both years the BLHs that fed on pine had the highest percentage (55 and 75%, respectively) of samples with beet curly top virus based on primers that can detect both the Worland and Colorado strains. Both years, BLH that had fed on Russian thistle and alfalfa had the highest percentage of samples with Spinach curly top Arizona virus. These data will be utilized in the development of future curly top management plans.

The management of beet leafhoppers (BLHs) is important for disease control since BLHs can vector important plant pathogens such as curly top viruses and phytoplasmas in southern Idaho. Historical data for southern Idaho suggests that BLH need approximately 130 growing degree days (GDDs; 12.8°C base) to initiate dispersal and around 382 GDDs until they reach peak dispersal. A recent study in southern Idaho identified large peak dispersal events of BLHs on May 19, 2020 and June 2, 2021 in Elmore Co. near Mt. Home, ID. Historically, BLH have been thought to originate from local areas. However, based on GDDs and dispersal numbers under optimal conditions for Mt. Home, the BLHs likely did not originate from local areas. Data for wind and pine pollen dispersal combined with GDDs for areas known to contain BLH suggest that the BLH could have originated outside the local area and possibly up to 142 to 515 km away. At least five conditions appear to be necessary for observation of BLH dispersal into southern Idaho: a wind event must occur (35 km/h avg. hourly wind speed), dispersal temperature threshold (16 to 18°C) must be met, >130 GDDs must be accumulated to initiate dispersal, daily peak temperatures should reach 24°C, and attractive BLH vegetation such as Russian thistle must be present. Combining wind event forecasts with temperature parameters in the future may make it possible to provide targeted timely insecticide sprays for BLH control.

**Caroline Toth,** New Mexico State University presented her research “**Feeding and oviposition preference of viruliferous beet leafhoppers among two non-host cover crops**”

Chile pepper (*Capsicum annuum* L.) yield in New Mexico is threatened by a multitude of factors, including beet curly top virus (BCTV) vectored by the beet leafhopper (*Circulifer tenellus* Baker) and competition with annual weeds. Barley (*Hordeum vulgare* L.) and brown mustard [*Brassica juncea* (L.) Czern.] may both be suitable cover crops for reducing early-season weeds in chile pepper; however, the possibility of attracting potentially viruliferous beet leafhoppers should be considered when selecting a cover crop. In this study, cafeteria-style dual choice tests were carried out between two barley cultivars (‘Stockford’ and ‘Valor’) and brown mustard (‘Caliente Rojo’). In a complementary follow-up to the cafeteria study, beet leafhoppers were subsequently trapped on individual leaves of barley and brown mustard, and the plant tissue was stained and examined for the presence of sheaths, punctures, and eggs. Beet leafhoppers demonstrated a preference for feeding on brown mustard that was nine times greater than preferences for either barley cultivar. When not provided with a choice, beet leafhoppers fed for longer periods of time on brown mustard than barley, as evidenced by the greater proportion of sheaths on the former. Additionally, no eggs were laid on either barley cultivar, indicating its unsuitability as a host plant. This research indicates that while neither cover crop is a host for BCTV, barley is less likely than brown mustard to sustain beet leafhopper populations that have the capacity to transmit the virus to subsequently planted chile pepper.

**Camille Wagstaff**, Washington State University presented her research “**Assessing seasonal host use and BCTV transmission by beet leafhopper in vegetable and hemp crop systems of the Columbia Basin**”

Pathogens such as Beet curly top virus (BCTV) and *Candidatus* Phytoplasma trifolii (CPt) are transmitted by insect vector beet leafhopper [*Circulifer tenellus*(Baker)] (Hemiptera: Cicadellidae), affecting many agricultural crops. Control methods for these pathogens 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, objective (1) linked pest monitoring and molecular tools to assess prevalence of leafhopper-transmitted pathogens across plant hosts, and objective (2) compared assessments of feeding habits of leafhoppers found in Washington potato crops using gut content analyses. Work involved sampling beet leafhoppers and associated host plants across Columbia Basin potato fields from 2019 to 2021. Plant tissue from sampling events was tested for leafhopper-transmitted pathogens, and leafhoppers were subjected to molecular gut content analysis to assess feeding patterns. Results indicate which hosts are widely used by leafhoppers during seasons and allow us to infer pathways of transmission among weeds and crops. 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. By understanding the timing of leafhopper migration and disease transmission, we can optimize the timing of pest management interventions.This research will promote education, extension outreach events, and multi-state collaborations which is essential. Overall, this research provides considerable information on the ecology of leafhoppers and associated pathogens and will aid in developing management tactics for these pathogens. More broadly, this work shows how molecular techniques can complement traditional monitoring efforts to better understand the dynamics of insect vectors and vector-borne plant pathogens.

**Kylie Swisher Grimm**, USDA-ARS Wapato, WA, presented her work on “**Mapping pathogen prevalence in beet leafhoppers collected from the Columbia Basin of Washington**”

In the Columbia Basin of Washington State, beet leafhopper-transmitted pathogens including Beet curly top virus, Beet leafhopper transmitted virescence agent phytoplasma, and *Spiroplasma citri* cause serious damage to vegetable and seed crops each year. Researchers with the USDA Agricultural Research service and Washington State University developed new tools to improve insect extraction and pathogen detection, enabling the high-throughput testing of field-caught beet leafhopper specimens for the three associated pathogens. These tools have enabled near real-time pathogen prevalence data to be generated for dissemination to potato and other vegetable and seed growers in the region using the Washington State University Potato Decision Aid System online tool. In addition, trends in pathogen abundance and crop type where beet leafhoppers were collected have been identified. Data generated each year has the opportunity to be incorporated into grower Integrated Pest Management programs, improving beet leafhopper management by either decreasing the cost and hazard of unnecessary insecticide sprays or increasing crop yield and quality by preventing disease outbreaks through well-time insecticide applications.

**Cynthia Ocamb,** Oregon State University, presented on her research on “**Curly top in hemp in Oregon and Washington”**

Hemp(*Cannabis* *sativa* L.) is an emerging crop in the Pacific Northwest, primarily produced for CBD extraction and smokable flower buds. Disease pressure faced by hemp in Oregon and Washington is not well understood so disease surveys were conducted at sites across Oregon and in the Yakima Valley of central Washington. Forty-three and 37 hemp growing sites were surveyed for diseases in 2021 and 2022, respectively. At each location, two 100-plant transects were examined shortly after transplanting as well as during mid- and late-season. Beet curly top virus (Mild/Worland strain) was confirmed by PCR in 16 and 18 Oregon hemp sites as well as in 10 and 8 Washington sites during 2021 and 2022, respectively. Symptoms were variable and included plant stunting, chlorosis, leaf distortion, leaf curling/twisting, leaf puckering, and upward curling of leaf tips. The frequency of plants symptomatic for virus infections was generally greater in sites in central Oregon, the Oregon portion of the Columbia Basin area near Hermiston, and Yakima Valley compared with hemp fields in the Willamette Valley and southern Oregon. A phytoplasma (Beet Leafhopper-transmitted Virescence Agent) and *Spiroplasma citri* also occurred in a subset of Oregon and Washington sites during both years of survey. Beet curly top virus-, phytoplasma-, and spiroplasma-infected plants were detected in field-grown and indoor hemp production sites; in some instances, all three pathogens co-occurred in the same hemp plant.

**Gina Angellela,** USDA-ARSWapato, WA presented her work on "**Exploring whether *Wolbachia* influences beet leafhopper feeding/probing behavior and beet curly top virus transmission**"

*Wolbachia*-infected and uninfected subpopulations of beet leafhoppers (Circulifer tenellus) co-occur in the Columbia Basin region of Washington and Oregon. While facultative endosymbionts such as *Hamiltonella defensa* have demonstrably altered feeding/probing behavior in hemipteran hosts, the behavioral phenotypes conferred by *Wolbachia* to its insect hosts, including feeding/probing, are largely understudied. We studied the feeding/probing behavior of beet leafhoppers from in-house colonies with and without *Wolbachia* on potato plants (*Solanum* *tuberosum* cv. Umatilla Russet) using electropenetrography. Insects were recorded for four hours, and wavelengths annotated following established conventions for beet leafhoppers. As both colonies carried beet curly top virus (BCTV), and some *Wolbachia* strains can mediate insect-vectored pathogen transmission, plants were maintained for two weeks post-assay and tested for successful BCTV inoculation. Waveform incidence and duration as well as BCTV transmission success were compared between beet leafhoppers with and without *Wolbachia*. Transmission rates did not vary; however, the frequency of phloem ingestion and the frequency and duration of phloem salivation increased with *Wolbachia* infection status in beet leafhoppers.

**Jinlong Han** in cooperation with **Punya Nachappa** Colorado State University presented his research on "**Unveiling the molecular mechanisms underlying the effects of beet curly top virus on beet leafhopper performance**"

Beet curly top virus (BCTV) is a major threat to sugar beets in the western US, spread exclusively by beet leafhoppers (BLHs, *Circulifer tenellus*). Despite progress in understanding virus-insect interactions, our understanding of the molecular interactions between BCTV and BLH remained unclear due to the limited omics resources for BLH. This study unveils the significant impact of BCTV on both the performance and transcriptome response of BLHs. Viruliferous BLHs had higher fecundity than non-viruliferous counterparts, which was evident by up-regulation of differentially expressed transcripts (DETs) associated with development, viability, and fertility of germline and embryos in viruliferous insects. Conversely, most DETs associated with muscle movement and locomotor activities were down-regulated in viruliferous insects, implying potential behavioral modifications by BCTV. Additionally, a great proportion of DETs related to innate immunity and detoxification was up-regulated in viruliferous insects. Viral infection also induced notable alterations in primary metabolisms, including energy metabolism namely glucosidases, lipid digestion and transport, and protein degradation, along with other cellular functions, particularly in chromatin remodeling and DNA repair. Our preliminary study of RNAi in BLHs showed 64% and 90% silencing efficiencies for gustavus and pumilio homolog 3 genes, respectively, paving the way for future functional analysis. This study represents the first comprehensive transcriptome analysis for BLH and confirms the feasibility of RNAi in BLHs for future functional analysis. Overall, the presented findings offer new understanding of BCTV's multifaceted effects on BLHs, providing a foundation for exploring the complex virus-vector relationship and developing potential management strategies for curly top disease.

**Max Schmidtbauer** in cooperation with **Jordan Withycombe, Jinlong Han,** and **Punya Nachappa** Colorado State University, presented his work “**A little help from my friend, virus infection reduces beet leafhopper susceptibility to neonicotinoid insecticide**”

Sugar beets are grown across the western United States for their lucrative taproot and are an economically important crop generating 1.16 billion dollars in 2021. However, production is threatened by the beet curly top virus (BCTV), a viral pathogen that severely reduces yield. The virus is exclusively transmitted by the beet leafhopper, Circulifer tenellus. Current management options for BCTV rely on neonicotinoid seed treatments and pyrethroid foliar sprays. However, there are reports of neonicotinoid resistance in numerous insect pests and this insecticide group may have harmful effects on non-target insects. Hence, the goal of this research is to examine susceptibility of beet leafhoppers to three different application rates (2x, 1x, 0.1x) of insecticides and determine their ability to lower virus transmission. We hypothesize that applying insecticide at a higher label rate will result in an increased mortality of beet leafhoppers, and therefore reduced virus transmission. At the 1x rate, neonicotinoids were more effective than pyrethroids, yielding 100% mortality of BCTV-infected and non-infected insects. However, at the 0.1x rate BCTV-infected leafhoppers had a higher survival rate than non-infected insects. This result was observed in only the neonicotinoid trials and not the pyrethroid trials. Using traditional bioassays and RNA sequencing technologies, we hope to confirm this potential insecticide resistance in BCTV-infected insects. This is an important piece of information to aid in tracking the epidemiology of BCTV and make predictions about leafhopper populations. With this knowledge, farmers would be able to adjust their management strategies to achieve more sustainable and profitable sugar beet production practices.

**Kendra Tapia**, CDFA BCTVCP presented her talk “**Beet Curly Top Virus Control Program Update**”

BCTV Program Update Summary 2024

* In September and October, BLH counts statewide remained low (0-3 per sweep). There was some nymph activity observed in the central districts, but it was minimal and isolated. No Fall operations were conducted for the 2023 year.
* In early Spring, rain and storm events limited the Program’s ability to conduct survey in the westside foothills. BLH counts remain low statewide (0-2 BLH per sweep). Fresno County had some high count areas in the hillsides (5-10 BLH per sweep set) but it remained isolated.
* In late spring, BLH counts remained low (0-3 BLH per sweep) statewide. Fallow field and roadside survey began. The central counties observed some nymph activity along roadsides. Russian Thistle, London Rocket, Mallow, Filaree, Goosefoot, Shepherd’s Purse, and Lamb’s Quarter accounted for the highest percentages of host plant vegetation.
* 160 acres were treated in Fresno County and 80 acres in Kings County in May due to high counts along roadsides (5-8 BLH per set range).
* The Program is discovering new host plants, and the host plant guide will be updated. We also have doubled the sampling process and will be partnering with new research contracts to help expand the Program knowledge and growth.

Various participants presented field perspectives from different states.

Idaho –Neonicotinoids have nearly eliminated the disease. Some late season infection

Colorado – Have found curly top on hemp and on leafhoppers on sticky traps. Worse on the Western Slope, but also on the Front Range.

Oregon – changes in heat/dry spells have had a big influence on BCTV.

California – Tom T. found BCTV-infected tomatoes in April in the Central Valley and on mustard cover crops. There has been major increases in fallow fields in the CV due to water shortages.

Tom tried using insecticide treatments (Ciazapure – Exirel) on tomato transplants. It lasts for around 4 weeks. He also has used a neonic (Assail) that appears to be less toxic to bees.

Washington – The trapping network is designed to collect around potatoes, but has resulted in very good prediction for curly top.

Research questions and priorities

Sugarbeet companies would like an RNAi solution to the curly top problem.

Breeding companies are still working toward breeding for resistance or tolerance to BCTV.

A non-traditional breeding approach might be the way to proceed.

What are alternative pesticides that could help with curly top?

 Can new technologies such as CRISPR or RNAi help with BCTV?

The 2025 WERA 1007 meeting will be held in Washington, with Kylie Swisher Grimm hosting. Possible dates are June 23-24, 2025

**Project Objectives:**

1. **Assess the current status of curly top and set priorities for integrated research on curly top disease.**
2. **Characterization of curtovirus strains including virus genetic diversity, new virus strains and virus in new hosts.**
3. **Organize research on the biology and ecology of the leafhopper, virus transmission, and the role of weed hosts in curly top in the western US.**
4. **Organize research to improve virus and vector management.**
5. **Provide a national platform for education on curly top disease, virus/insect/plant ecology and management, collaboration among scientists involved in these activities, and extension of research-based information for producers.**

**Objective 1:**  Accomplished through annual meeting presentations and goal setting. See above minutes.

**Objective 2:** Worked toward this goal in individual research programs. See above minutes.

**Objective 3:** Made progress toward the goal. See above minutes.

**Objective 4:** Made progress toward goal. See above minutes.

**Objective 5:** Collaborative curly top projects were carried out between Carl Strausbaugh and Kylie Swisher Grimm and between Punya Nachappa, Oliver Neher, Carl Strausbaugh, and Raj Majumdar

**Impact Statement**

Curly top is an economically important disease in many states in the western U.S. Members of the WERA1007 group increased the knowledge of the virus biology, its transmission, and the management of the disease. The range of virus strains was expanded in California, Colorado, Washington, and New Mexico. The resistance to curly top in sugarbeets was assessed, aspects of the curly top virus transmission were characterized, and the use of foliar insecticides for vector control was tested. These findings should help improve the management of curly top in sugar beet and other affected crops in the western U.S.

**Publications**

The group did not publish a report together. The following curly top related publications were published during the last year:

Strausbaugh, C.A., Wenninger, E.J., Jackson, L.K., Vincill, E.D. 2024. Host and shelter plants for the beet leafhopper which vectors curly top viruses and phytoplasmas in southern Idaho. PhytoFrontiers. <https://doi.org/10.1094/PHYTOFR-03-24-0022-R>.

Strausbaugh, C.A., Wenninger, E.J., Jackson, L.K., Vincill, E.D. 2024. Curly top viruses and phytoplasmas in sugar beets, common beans, and beet leafhoppers along with vector population dynamics in southern Idaho. PhytoFrontiers. 4:1-14. <https://doi.org/10.1094/PHYTOFR-08-23-0115-R>.

Han, J., Cui, M., Withycombe, J., Schmidtbauer, M., Chiginsky, J., Neher, O., Strausbaugh, C., Majumdar, R., Nalam, V.J., Nachappa, P. 2024. Beet curly top virus affects vector biology: The first transcriptome analysis of the beet leafhopper. J. Gen. Virol. In press.

Foutz, J.J., Cooper, W.R., Swisher Grimm, K.D., Crowder, D. 2024. Seasonal and lifecycle changes in behavior affect the trapping efficiency of an insect vector, Circulifer tenellus (Hemiptera: Cicadellidae). Annals of the Entomological Society of America. 117(3):199-205. https://doi.org/10.1093/aesa/saae011.