**Molecular Insights into Host Use and Pathogen Acquisition by an Insect Leafhopper Vector in Potato Crop Fields**

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**Abstract:**

Insect vectors are notoriously difficult to manage due to their ability to utilize a wide range of host plants across seasons. Improving vector management requires novel approaches that assess host use across space and time to predict pathogen transmission dynamics. Molecular gut content analysis of vector insects has been instrumental in identifying host use but is often limited by its inability to directly link hosts to pathogen transmission. Here, we integrate gut content analysis with pathogen incidence to determine the role of various host plants in *Neoaliturus tenellus* (previously *Circulifer tenellus* Baker) (Beet leafhopper: *Hemiptera: Cicadellidae*)movement and pathogen spread. We tested 230 leafhopper adults collected from 15 sites over three years to assess host utilization and pathogen acquisition. Our results confirm that *N. tenellus* acquire pathogens from *Sisymbrium* spp. and *Brassica* spp. (wild mustards) in the spring, while *Salsola/Kali* spp. (Russian thistle) and *Bassia* spp. (kochia) serve as primary *N. tenellus* hosts throughout the summer. A new discovery, we detected gut content from trees, including *Tilia* spp. (linden), *Prunus* spp./*Pyrus* spp. /*Citrus* spp (fruit), and *Tsuga* spp./*Pinus* spp, (pine) suggesting previously unknown host interactions. These findings refine our understanding of vector ecology and highlight the importance of host use patterns in predicting pathogen transmission, ultimately improving risk assessments and integrated pest management strategies.

**Keywords:** gut content analysis, beet curly top virus, purple top disease, phytoplasma, landscape ecology, pest population dynamics, vector monitoring

**Introduction:**

Many insect pests that are vectors of pathogens are generalists that feed on diverse hosts across landscapes and seasons (Gutiérrez-López et al. 2020; Weintraub and Beanland 2006). Generalist vectors are notoriously difficult to manage due in part to their ability to move between different crop types and non-crop weedy hosts (Gutiérrez Illan et al. 2020; Heck 2018; Nault 1997). Areawide management is challenging to coordinate, and large patches of weedy hosts are often unmanaged nearby commercial production areas, providing sources of vector populations at variable points during crop seasons (Bennet 1971). Pathogens transmitted by vectors also have variable ability to attack different host species, and these infectivity traits may not always align with vector host preferences (Thapa and Ghersi 2023). A comprehensive understanding of host use by vectors and pathogens is key for predicting vector and pathogen dynamics and forecasting disease outbreaks while also aiding in developing mitigation strategies.

Effective vector management requires innovative approaches to assess host use over space and time to better predict pathogen transmission dynamics (Rafter and Walter 2020). One emerging technique to assess insect host use is molecular gut content analysis, which identifies ingested plant DNA within insect digestive systems to reconstruct trophic interactions (Cooper et al. 2019; Cooper at al. 2022; Pitt et al. 2024). When molecular gut content analysis is combined with structured surveys of insects across broad regions, the technique can be used to infer patterns of insect movement across broad landscapes within and across seasons (Cooper et al. 2019; Strausbaugh et al. 2024). While gut content analysis cannot directly establish a link between vector feeding events and pathogen acquisition, integrating gut content analysis with pathogen incidence data could clarify the relationships between host use and pathogen spread.

The ecology of many insect vectors, such as the beet leafhopper (*Neoaliturus tenellus* Baker; Hemiptera: Cicadellidae), is often largely based on natural history surveys or structured surveys of agricultural systems (Brewster and Allen 1997; Damos 2015; Wohleb et al. 2021). However, for many vectors, the plant hosts that serve as pathogen reservoirs, and thereby pose the greatest risk to crops, are largely inferred rather than directly known (Horton et al. 2018). For example, it may often be assumed that most hosts used by a vector will also be hosts for pathogens transmitted by that vector, or that hosts will be a predictable subset constrained by plant phylogeny (Perilla-Henao and Casteel 2016). Molecular tools are an effective way to bridge this knowledge gap, as the same insects can be tested for the pathogens they carry as well as their prior host use (Cooper et al. 2019; Cooper at al. 2022; Pitt et al. 2024).

Here, we leveraged molecular gut content analysis to trace landscape-scale movements of *N. tenellus* in a major potato production region of the United States, the Columbia River Basin region of Washington State. As the sole vector of *Beet curly top virus* (BCTV), *Candidatus* Phytoplasma trifolii (CPt) (previously known as Beet leafhopper-transmitted viresence agent (BLTVA), and *Spiroplasma citri* (*S. citri*) in the Columbia River Basin, *N. tenellus* facilitates pathogen spread across cropping areas throughout the season (Cooper et al. 2023). Previous monitoring suggests that wild Brassicaceae species serve as overwintering reservoirs, while Amaranthaceae species support leafhopper populations during the growing season (Horton et al. 2018). However, it is still relatively unknown which non-crop and crop hosts are most associated with these particular pathogens. Over three years, we analyzed 230 adult *N. tenellus* collected from 15 sites across the Columbia Basin to determine seasonal host use and pathogen incidence. This knowledge can enhance targeted control measures and mitigate crop losses associated with leafhopper-borne pathogens and associated crop diseases.

**Materials & Methods:**

***Study system***

Leafhopper-transmitted pathogens threaten many crops in the western United States, including potatoes and seed crops such as sugar beet, carrot, spinach, hemp, sunflower, and coriander (Hudson et al. 2010; Munyaneza et al. 2006a; Nachappa et al. 2020; Rondon and Murphy 2016; Soto and Gilbertson 2003). Gut adaptations of *N. tenellus* allow pathogens to traverse the stomach lining and colonize within salivary glands, facilitating highly efficient transmission (Knowlton 1929; Suzuki et al. 2006; Frantz et al. 2023). Further, *N. tenellus* does not exhibit strong host specificity, often feeding on hosts that cannot fully support development, which enhances its ability to spread pathogens across diverse hosts (Thomas and Boll 1977). In turn, the persistence and annual resurgence of pathogens are closely linked to the life cycle and migratory behavior of *N. tenellus*. Overwintering females lay eggs in spring, and nymphs acquire pathogens from weedy host plants before dispersing into agricultural fields (Lee et al. 2022; Meyerdirk and Hessein 1985; Meyerdirk and Moratorio 1987). Early-season populations tend to establish on weeds in the Brassicaceae family, which serve as primary hosts in spring (Hudson et al. 2010). As temperatures rise in mid-April and these weed hosts dry out, *N. tenellus* disperse into summer crops (Horton et al 2018; Munyaneza et al. 2006b). This seasonal movement plays a role in the epidemiology of BCTV and CPt, yet the full extent of host use and pathogen transmission across seasons remains poorly understood. To better characterize seasonal host use of *N. tenellus* and its role in pathogen transmission, we conducted molecular gut content analysis across three growing seasons.

***Site Selection and Sampling***

Sampling was conducted at sites across the Columbia River Basin during the 2019 and 2020 growing seasons and in spring of 2021 (Fig. 1). Most sites were included in the Washington State University’s potato pest monitoring network, a standardized sampling network on commercial farms; additional sites were at university research farms. In 2019 and 2020, sampling focused on irrigated potato fields and adjacent weedy areas, while in 2021 collections occurred near potato fields before planting (Fig. 1). Our approach aims to identify key plant species that may serve as hosts at different times of the year, and plant tissue samples from collection sites were tested for the presence of pathogens to assess potential sources of infection (Foutz et al. 2025). Linking vector molecular gut content analysis with pathogen detection may resolve host-pathogen interactions and improve predictive models for disease outbreaks (Cooper et al. 2019). Sampling occurred every other week in 2019 and 2020 and weekly in 2021. Insects were collected using a reversible leaf blower, suctioned into organdy bags, and transferred to resealable plastic bags (26cc Gas Handheld Blower Vacuum, Homelite Corporation, Charlotte, NC). All potential weedy host stands within 100 m of potato fields were sampled for one minute per plant stand. Additionally, five leaves per sampled stand of weeds or potatoes were collected for pathogen testing. Live insects and plant samples were transported on ice and immediately frozen at -40 °C upon arrival at the laboratory. Identification and sexing were performed under a stereo microscope using distinct morphological traits of tapered, bullet body shape measuring 3 to 4 mm in length, with a pale green to tan coloration, and specimens were stored in a -40 °C freezer.

Of the 1,765 adult *N. tenellus* collected across the Columbia Basin from 2019 to 2021 in this manner, 230 *N. tenellus* from potato fields were selected for gut content analysis to assess seasonal host use and pathogen transmission dynamics. Initially, we surveyed infection status based on the host plant species from which leafhoppers were collected. In 2019, a total of 64 *N. tenellu*s individuals were selected from kochia, potato, russian thistle, pigweed, and mustards. This sub-sample revealed minimal infection incidence, limiting our ability to detect viable host reservoirs. Consequently, in 2020 a total of 93 *N. tenellu*s individuals were selected based on infection status. Gut content analysis was performed on 24 BCTV-infected leafhoppers, 25 *C*Pt-infected leafhoppers, 23 leafhoppers co-infected with both BCTV and CPt, and 25 healthy or non-infected leafhoppers. Current hypotheses are that leafhoppers obtain pathogens from weeds used in overwintering and that the greatest threat to crop infection may be in spring as leafhoppers transition from overwintering hosts to new crop growth (Horton et al. 2018). To explore the validity of the spring migration hypothesis we focused our sampling in 2021, to include 73 individual beet leafhoppers collected in spring and early summer.

***DNA Extraction***

Adult *N. tenellus* were surface sterilized by sequentially immersing them individually in 70% ethanol for 5 seconds, sterile deionized water for 5 seconds, followed by a 60-second treatment in 1% bleach, and two final 5 second rinses in sterile deionized water. Specimens were then air-dried on Kimtech Science™ Kimwipes™ placed within a sterile petri dish. Total DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. All extraction procedures were carried out in a UV-sterilized biosafety cabinet to prevent contamination. To assess DNA quality and concentration, a subset of the extracted samples was evaluated using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, MA). Negative controls lacking DNA templates were included throughout the extraction and analysis process to verify that no contamination was present in the reagents. All extracted DNA samples were stored at -20 °C until further molecular procedures were carried out.

***Pathogen Identification***

Testing for the presence of *Ca*. P. trifolii (CPt) in insects was done using real-time PCR on a Lightcycler 480 (Roche, Basel, Switzerland) with these conditions: a 5 min hold at 95 °C, 20 cycles of 95 °C for 10 sec, 65 °C for 10 sec, and 72 °C for 10 sec, then 20 cycles of 95 °C for 10 sec, 55 °C for 10 sec, and 72 °C for 10 sec, a melting curve to assess primer specificity, and a cooling cycle. Each 20 μL reaction contained 10 μL of SYBR™ Green PCR Master Mix (ThermoFisher Scientific, Waltham MA), 8.2 μL of nuclease-free water, 0.4 μL each of *Ca*. P. trifolii primers “z-R16R2-wfB\_F” (AAA TAT TTC TCG GGG TTT GTA CAC ACC GCC CGT CA) and “BLTVA-int-wfB\_R” (AAT TAT CTC TGA TGA TTT TAG TAT ATA TAG TCC) at 20 μM concentration, and 1 μL of extracted *N. tenellus* DNA (Cooper et al. 2023, Swisher Grimm et al. 2023).

Testing for the presence of *Beet curly top virus* (BCTV)in samples was by conventional PCR on a BioRad thermocycler with these conditions: 1 min at 95 °C, 20 cycles of 95 °C for 15 s, 65 °C for 30 s (touchdown, Δ−0.5 °C), and 72 °C for 20 s, then 20 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 20 s, then 1 min at 72 °C before an infinite hold at 4 °C until samples were removed. Each 20 μL reaction contained 10 μL of Amplitaq Gold 360 Master Mix (ThermoFisher Scientific, Waltham, MA), 8.6 μL of nuclease-free water, 0.2 μL each of primers “BCTV2-F” (GTG GAT CAA TTT CCA GAC AAT TAT C) and “BCTV2-R” (CCC ATA AGA GCC ATA TCA AAC TTC) at 20 μM concentration, and 1 μL of extracted *N. tenellus* DNA (Strausbaugh et al. 2008, Swisher Grimm et al. 2023). Infection was confirmed by visualizing approximately 520-bp PCR products under UV light on a 1% agarose gel stained with GelRed (Biotium, Fremont, CA).

Samples from 2019 and 2020 were also tested for the presence of the bacterium *Spiroplasma citri*, another plant pathogen transmitted exclusively by *N. tenellus*. Testing for the presence of *S. citri* was done using conventional PCR with conditions identical for *Beet curly top virus* identification listed above. Each 20 μL reaction contained 10 μL of Amplitaq Gold 360 Master Mix, 8.6 μL of nuclease-free water, 0.2 μL each of primers “S.citri-1” (GGT CTG CTG CTT TAA TTT CTA C) and “S.citri-2” (TGC AGC ACC TGC AAC TGT AG) at 20 μM concentration, and 1 μL of extracted *N. tenellus* DNA (Cooper et al. 2023, Swisher Grimm et al. 2023). *S. citri* infection was determined by visualizing the approximately 350-bp PCR products under UV light on a 1% agarose gel with GelRed staining. Only 8% (8/97) of tested samples showed infection of *S. citri*. Due to low infection levels, *S. citri* was left out of further analysis. For a collaborative project looking into *N. tenellus* populations and migrations, 51 of the 2020 *N. tenellus* were sequenced using Restriction site-associated DNA sequencing (RAD-seq) (unpublished Gina Angelella).

For molecular pathogen detection in this study, we used Real-time PCR, and not quantitative PCR. This approach was chosen as presence/absence data was sufficient to address the study’s research objectives. DNA concentrations were not standardized across samples; therefore, Cq values are not reported, as they do not accurately reflect pathogen titers in insect tissues (Ruiz-Villalba et al. 2021).

***Molecular Gut Content Analysis***

The dietary profiles of *N. tenellus* were investigated using high-throughput, single-molecule real-time (SMRT) sequencing on the PacBio sequencing platform. Plant-derived DNA was amplified from individual insect gut extractions using primers targeting two common plant barcoding loci: the chloroplast trnF region and the nuclear internal transcribed spacer 2 (ITS2). PCR amplification was conducted separately for each locus using universal primers: trnF (B49873-e: GGTTCAAGTCCCTCTATCCC; A50272-f: ATTTGAACTGGTGACACGAG; Taberlet et al., 1991) and ITS2 (ITS2F: ATGCGATACTTGGTGTGAAT; ITS3R: GACGCTTCTCCAGACTACAAT; Chen et al., 2010). To enable numerous samples to be sequenced in the same pooled set, each sample was assigned a unique combination of asymmetric barcoded forward and reverse primers (Pacific Biosciences, 2014) as described in Cooper et al. 2019 and 2022. Reactions were performed in 50 µL volumes, using 40uL Invitrogen Amplitaq Gold 360 PCR Master Mix at 62.5% (Invitrogen, Carlsbad, CA), 250 nM of each primer (or 5 µmol/L of forward and reverse primers), and 5 µL of DNA template.

Thermocycler conditions for PCR of ITS2 included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, and 72 °C for 45 seconds, with a final extension of 5 minutes at 72 °C. For trnF, cycling conditions were slightly adjusted: an initial denaturation at 94 °C for 10 minutes, 40 cycles of 94 °C for 30 seconds, 58 °C (or 52 °C for some optimized reactions) for 30 seconds, and 72 °C for 45 seconds, followed by a 5-minute final extension at 72 °C. Amplicon sizes approximately 500 bp were confirmed through visualization on electrophoresis 1% agarose gels stained with either ethidium bromide or SYBR® Safe (Thermo Fisher Scientific).

The remaining PCR product volumes for pooling were adjusted based on band intensity, and PCR products were cleaned using QIAquick® PCR Purification Kits (Qiagen, Hilden, Germany) prior to pooling. Each pooled sample set included no-template controls (NTCs) containing water and positive controls consisting of psyllid species collected directly from feeding on known host plants, each with their own unique barcode set to monitor contamination and PCR efficiency. The pooled library was concentrated using AMPure XP beads (Beckman Coulter), end-repaired, ligated to SMRTbell adapters with the Express Template Prep Kit v2.0, and quantified prior to sequencing on 1M v3 SMRT cells using the Sequel Binding Kit 3.0. The run was conducted for 10 hours at the Washington State University Laboratory for Biotechnology and Bioanalysis Genomics Lab. Raw sequence data were processed using SMRT Link v6.0 to generate high-quality circular consensus sequences (CCS). Demultiplexed reads were filtered to retain only sequences between 400 and 700 bp and with a minimum quality threshold (Phred ≥ 40, inferred accuracy ≥ 0.9999). Sequence data were analyzed using Geneious Prime® (v2023.1.2). Operational taxonomic units (OTUs) were generated via de novo assembly using custom parameters (95% minimum overlap identity, 1% max gaps per read, and 5% max mismatches). OTUs represented by five or fewer reads were excluded to minimize the inclusion of artifacts or potential contamination. BLASTn searches against the NCBI GenBank database were used to assign taxonomic identities, with matches reported to the genus level, not species, for greater reliability (Altschul et al. 1990). A plant taxon was considered present in a sample if at least six reads matched a given OTU, a threshold consistent with previous metabarcoding studies (Cooper et al., 2022). Given that prior research has shown plant DNA signal intensity may not correlate with feeding intensity or time since ingestion, dietary results were interpreted qualitatively as presence/absence data (Avanesyan et al., 2021).

***Analytics***

Data was evaluated to explore seasonal trends in *N. tenellus* infection status and variation in host plants found in guts across the Columbia River Basin. The observational nature of this study precluded formal hypothesis testing. Instead, we present a general summary of our surveys to show the variability in infection status, the diversity of host plants used, and the seasonal timing of host plant use by *N. tenellus*. We considered that variation in host plant species composition may explain differences in infection status and made these comparisons with PERMANOVA (*vegan::adonis2*) (Oksanen et al. 2024). This approach takes the matrix of species composition as a function of BCTV infection status, CPt infection status, collection region, and collection year as fixed effects and the host plant species that each *N. tenellus* was collected on as a random intercept. To explore the hypothesis that *N. tenellus* seasonally migrate from weeds to crops in early spring we included a spline term (*df* = 5, knots = 0) for the sampling date of each *N. tenellus* in our PERMANOVA. Significant effects were evaluated by constrained ordination via Canonical Analysis of Principal Coordinates (CAP), a robust method for parsing out complicated effects in multivariate analyses without Euclidean distance constraints (Anderson & Willis 2003). The effect of sampling date was visualized by fitting a generalized additive mixed model (GAMM) surface overlay with smooth terms for the constrained (CAP) and unconstrained (MDS) axes (*df* = 5) using the *gamm4* package (Wood & Scheipl, 2020). All multivariate analyses were restricted to the top tenth percentile of species based on abundance. As an alternative approach, we used kernel density estimation and the *ggridges* package (Wilke 2024) by aggregating the occurrence of each host plant record in *N. tenellus* guts across all samples. These counts were then aggregated by sampling date and kernel density estimation using Silverman’s rule (Gaussian smooths for small sample size) was used to visualize trends in host plant use over time (Scott 1992). All analyses were conducted in R v 4.2.3 (R Core Team, 2023) and the *tidyverse* ecosystem (Wickham et al. 2019).

**Results:**

Overall, we collected 1,765 adult *N. tenellus* across three years throughout the Columbia Basin and analyzed the gut contents of 230 (13%) paired with pathogen analysis for three of their vectored pathogens. Of the 230 *N. tenellus*, 113 were healthy, 39 were infected with only BCTV, 43 were infected with only CPt, and 27 were co-infected with BCTV and CPt. Only the *N. tenellus* collected in 2020 were also tested for *S. citri,* with 8 out of the 93 tested being positive; 5 were infected with just *S. citri,* 1 co-infected with both BCTV and *S. citri,* 1 co-infected with both CPt and *S. citri,* and 1 co-infected with all three pathogens. After removing likely erroneous barcoding identifications, gut content analysis revealed 2,435 total plant hits, representing 246 plant species, 150 plant genera, and 45 plant families. The majority of hits at 1,484 were non-crop weeds (61%), mainly *Bassia*, *Salsola*, *Sisymbrium*, *Amaranthus*, and *Kali spp.* (Fig. 2). Out of all plant hits the percentages were highest for various mustards (17.9%), Russian thistle (11.3%), kochia (8.6%), nightshades (5.3%), pigweed (2.7%), lambsquarter (2.6%), and a mixture of other Asteraceae and Amaranthaceae species (12.5%). Crops were also found in *N. tenellus* guts, primarily *Raphanus*, *Cannabis*, *Medicago*, and *Vigna* (Fig. 2). Surprisingly, we also detected multiple tree species, especially *Tilia* spp. or linden trees (Fig. 2). While most *N. tenellus* were healthy, infection status (BCTV, CPt, and Co-infection) was similar across plants found in gut contents, with infection status varying independently among crop types (*F*6 = 0.89, *P* = 0.50).

Plant community composition in *N. tenellus* guts varied independently of CPt infection status (*F1* = 0.72, *P* = 0.676, R2 < 0.01) and region (*F*4 = 1.08, *P* = 0.613, R2 = 0.01). BCTV infection status had a significant but weak effect on composition (*F*1 = 3.95, *P* = 0.002, R2 = 0.01). Permutation tests for homogeneity of multivariate dispersions ruled out a false positive by heterogeneous dispersions (*F* = 1.41, *P* = 0.216, permutations = 999). Given the de minimis effect size of BCTV infection status, we visualized this effect by constrained ordination (CAP) of the variation explained by BCTV which represented only 1.0% of the total variation in gut content composition. In comparison, the first unconstrained axis (MDS1) captured 24.1% of the residual variation (Fig 3). Plant species vectors align primarily with variation in the unconstrained axis, orthogonal to the constrained BCTV axis, further highlighting the negligible effect of community separation by BCTV infection. Gut content plant community composition varied significantly over sampling date (*F5* = 10.20, *P* < 0.01, *R*2 = 0.16) (Fig. 3), however, this effect was collinear with collection year. CAP analyses aligned with the biases in our sampling design between sampling timing and year. Kernal density estimation provided complimentary results, in that variation in gut plant community varies over time. Peak detection in gut contents generally favored non-crop weeds earlier in the season (Fig. 4A), with crops detected slightly later in the season (Fig. 4B). Importantly, density plots (Fig. 4) with large magnitudes and multiple sharp peaks represent plants with low sample sizes, making the true seasonal distribution in *N. tenellus* guts uncertain.

**Discussion:**

This study provides a preliminary view of *N. tenellus* host use across seasons and their relationship to pathogen transmission, leveraging novel integration of gut content analysis and pathogen testing. Our results offer a first look into the diversity of plants consumed by *N. tenellus* in the Columbia Basin. Identifying seasonal host plants of *N. tenellus* can aid in vector movement predictions, reduce the risk of pathogen transmission to economically important crops, and inform targeted management strategies. Gut contents of *N. tenellus* were primarily composed of non-crop weeds, with non-crop abundance typically one order of magnitude greater than crop or tree abundance. Across all samples, *N. tenellus* individuals fed on a median of five plant species, four genera, and three families, indicating relatively high host breadth. However, there was considerable variability among individuals, with some feeding on as many as 20 different species and others on only one. The standard deviation was highest at the species level (3.92), reflecting greater heterogeneity in species-level feeding. This suggests that while some leafhoppers are highly polyphagous, others may exhibit more restricted feeding behavior. Understanding this variation in host use is crucial for identifying key transmission windows and plant species that may serve as bridges for BCTV and CPt spread. However, plant species in the guts of *N. tenellus* were not defined by infection status with BCTV or CPt. That is, gut plant communities were similar regardless of pathogen status. This finding may represent broad dietary generalism coupled with uniform pathogen dispersion at the landscape scale. However, we believe an equally plausible explanation is that hypersensitivity in the molecular tools used to identify gut contents may have detected trace amounts of ubiquitous plant residue, biasing our results. For example, at the time of sampling, many of the most common weeds (*Kali*, *Bassia*, *Salsola*, *Sisymbrium*) appeared in 89.9% (205/228) of the *N. tenellus* guts assayed. Whether this result is representative of their actual diet, or an artefact of environmental contamination demands further investigation. Given this alternative hypothesis, we reason that shifts in dietary composition with BCTV or CPt infection may still exist and could be a potential driver of vector behavior and host selection, regardless of confirmation from controls and our negative results.

By sampling from early spring to late summer, our surveys uniquely capture the seasonal variation in host use of *N. tenellus*. This seasonal variation lends support for the spring migration hypothesis in that *N. tenellus* may migrate from early emerging non-crop weeds to crops throughout the summer. In early spring, gut contents were marked by peak abundance of non-crop weeds such as *Bassia*, *Descurainia*, *Sisymbrium*, and *Lycium*. Most crop species (*Cannabis*, *Raphanus*, *Medicago*, and *Cucumis*) were found in peak abundance in late spring to early summer. Some plants were found in *N. tenellus* guts throughout the season at near uniform distributions. Notably, these ubiquitous all-season plants, *Salsola* and *Kali* may represent reservoirs for continuous reinfection throughout the growing season. Further evidence of host plant species turnover throughout the season was found in our multivariate results, with sampling date representing the majority of variation in species composition that could be explained by our predictors. While we are confident that there exists substantial variation in host use throughout the season, our experimental design partially confounds seasonal effects with year effects, therefore, these seasonal descriptions of potential host plant use should be considered preliminary.

Our results suggest that wild mustards, particularly *Sisymbrium* and *Descurainia* species, may serve as primary spring hosts for *N. tenellus*, with peak mustard feeding coinciding with the early-season emergence of nymphs (Foutz et al. 2025). These hosts are essential for *N. tenellus* development and early pathogen acquisition. Mustards are likely key spring hosts due to their ability to overwinter, cold hardiness, and tendency to germinate earlier than many other weed species (Cici and Van Acker 2011). Interestingly, our study also uncovered consistent detection of tree species—including *Tilia*, *Pyrus*, *Prunus*, and conifers like *Pinus* and *Tsuga*—in gut content, suggesting that trees may play a previously unrecognized role in *N. tenellus* seasonal ecology. Other research using DNA barcoding has also identified unexpected host plants, including coniferous trees such as *Pinus* spp. in the diet of *N. tenellus*, particularly during early summer dispersal events (Strausbaugh et al. 2024). These findings further support the idea that leafhoppers may feed on a broader range of plant taxa—including trees—than previously recognized, which has important implications for understanding their movement ecology and the epidemiology of their vectored pathogens. (Strausbaugh et al. 2024). While these data may suggest feeding, it is also possible that these detections reflect external contamination from environmental sources like pollen, rather than true host plant ingestion. Nonetheless, these findings highlight the importance of carefully interpreting molecular gut content data, particularly when unexpected taxa are detected.

Although, the upward migration to trees during spring bolting of mustards suggests trees could serve as temporary or transitional hosts. This pattern of host switching driven by plant phenology may highlight the importance of considering perennial hosts in disease surveillance and management programs. As mustards begin to bolt and turn yellow in spring, they become detectable via satellite imagery, providing an independent line of evidence that correlates with observed patterns of *N. tenellus* emergence and migration timing (Lee et al. 2025). This phenological cue, visible at landscape scale, suggests that satellite-based monitoring could be used as a predictive tool for vector movement.

We also confirmed strong seasonal patterns in host use. Kochia (*Bassia* spp.) emerged as a major summer host and pathogen reservoir, with infections persisting into the fall, while Russian thistle (*Salsola* spp.) was the most frequently detected genus overall. Seasonal host shifts appear driven by temperature and host availability, with nymphs showing localized feeding on preferred spring hosts and adults dispersing to irrigated or overwintering hosts in summer and fall (Horton et al. 2018). Interestingly, while plants like *Salsola* were commonly detected in gut contents and fed on by pathogen-positive *N. tenellus*, a complementary study showed the plants themselves exhibited low pathogen infection rates (Foutz et al. 2025). This decoupling between vector infection and plant infection highlights complexities in the transmission process and raises questions about vector feeding behavior, host quality, and virus retention. These results suggest that not all frequently visited or consumed plants contribute equally to pathogen amplification and that some may act more as *N. tenellus* hosts than as true pathogen reservoirs.

A particularly revealing finding was the negative association between BCTV infection and gut richness: *N. tenellus* individuals infected with BCTV had significantly fewer plant species in their guts. In contrast, CPt infection showed no relationship to host richness. This divergence likely reflects differences in pathogen biology or seasonality. BCTV requires replication in host plants, potentially encouraging vectors to remain on infected hosts and feed on fewer plants, whereas phytoplasmas are known to proliferate inside *N. tenellus*, reducing pathogen dependence on infected plants(Alkhatib et al. 2024; Koinuma et al. 2020). These results support the hypothesis that viruliferous insects exhibit altered behavior, potentially reducing movement and increasing feeding on infected plants to acquire a higher virus load, facilitating efficient BCTV transmission (Han et al. 2024).

While these findings offer important insights, several limitations must be acknowledged. Sampling was not uniform across years. In 2019, sampling was focused on fall, while sampling in 2021 emphasized spring collections, potentially biasing seasonal comparisons. The observed trend of BCTV-infected *N. tenellus* having fewer plant species in their gut contents could be linked to the timing of their collection. In the spring, *N. tenellus* nymphs hatch and develop on infected mustards, remaining relatively stationary until rising summer temperatures kill their preferred weed hosts, forcing migration into irrigated crops (Horton et al. 2018). By fall, *N. tenellus* begin seeking shelter and identifying suitable overwintering hosts (Horton et al. 2018). This search for an overwintering host may drive more selective feeding behavior, potentially leading to a greater diversity of plants detected in gut content analyses.

Gut content analysis may have potential limitation due to primer specifity of the DNA barcoding primers used, which may bias results toward certain genera. To address this, we used two primer types, ITS2 for broad taxonomic identification and trnF for targeting specific weed species. Additionally, we validated results at the genus level to account for potential sequencing errors and DNA degradation due to digestion and UV damage. These methodological refinements enhance the accuracy of our findings and strengthen our interpretations of *N. tenellus* feeding ecology and pathogen transmission. Despite these precautions, some low-abundance hosts may be underrepresented. Additionally, infection detection represents a snapshot in time, and our data cannot distinguish between transient and persistent infections.

In the broader context, our research improves understanding of how vector ecology drives disease risk and transmission dynamics in specialty cropping systems. By identifying key host species, seasonal trends, and differences in pathogen-vector interactions, we lay the groundwork for more precise and adaptive management strategies. Expanding this work to include real-time monitoring, deeper pathogen sequencing, and transmission trials could further clarify the roles of host plants and vector behavior in shaping disease dynamics. Incorporating our findings into broader integrated pest management frameworks offers opportunities to reduce pesticide use, lower grower costs, and promote long-term agricultural sustainability.

The economic impact of this research is substantial. Growers currently rely on intensive insecticide applications, costing approximately $400 per acre annually, to mitigate leafhopper-borne diseases (Galinato 2020). By refining our understanding of *N. tenellus* movement and feeding behavior, we can enhance predictive models and decision-support systems, such as those already in use for potato pest management, potentially reducing unnecessary pesticide applications and saving growers millions of dollars annually. Our findings can support more effective and sustainable pest control measures with demonstrated economic impact and are part of a larger monitoring network through Washington State University’s Decision Aid System (DAS), which provides precision pest management recommendations and has already saved potato farmers $9 million annually. Expanding this system to specialty crops, like vegetable seed, tomato, and pepper, and hemp crops, could generate similar economic benefits while promoting sustainable pest management practices and reducing pesticide use.

**Figure Legends:**

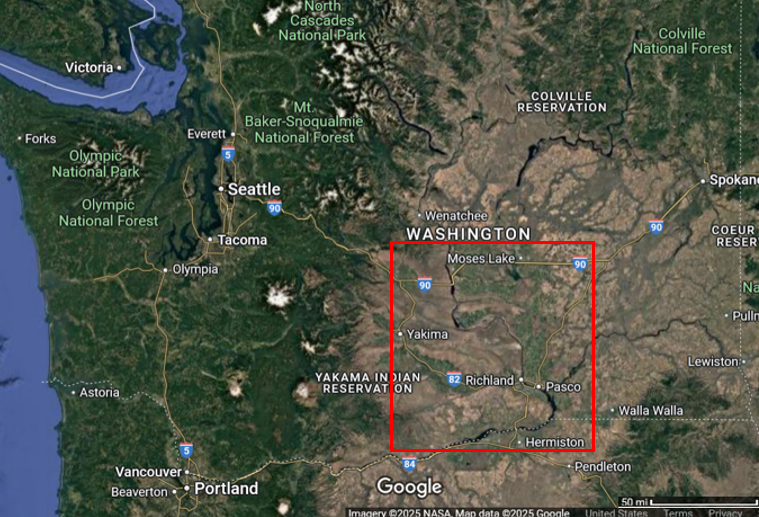
**Figure 1.** Map of study sites using GPS coordinates for where *N. tenellus* were collected from each year.

**Figure 2. *Neoaliturus tenellus* Gut Content Analysis.** Counts of plant genera detected in individual *N. tenellus* gut contents, categorized into three groups: crops, non-crop or weeds, and trees. The x-axis represents the count of leafhoppers in which each plant type was detected, while the y-axis lists the plant genera identified. The legend indicates infection status, with co-infection referring to the presence of both *Candidatus* Phytoplasma trifolii (CPt) and Beet Curly Top Virus (BCTV). (*S. citri* identification was excluded from analysis due to low frequency of results).

**Figure 3. Canonical analysis of Principal Coordinates for gut contents.** CAP1 represents all variation in gut content plant community composition constrained to show separation by BCTV infection status. MDS1 represents the first major axis of residual (unconstrained) variation in community composition. Points are individual *N. tenellus* gut assays. The direction of species vectors indicates increases in relative abundance across the ordination space, while the length of the vector indicates the strength of correlation between each species and variation in ordination space. Vectors aligned with CAP1 would indicate collinearity with BCTV infection. Vectors orthogonal to CAP1 indicate species whose abundances are primarily independent of BCTV infection. Grey isoclines represent sampling date across the growing season and are generated by a GAMM surface overlay. Numbers on isoclines are the day of year from 1 January.

**Figure 4. Seasonality of *Neoaliturus tenellus* Gut Content Analysis.** Genus-level seasonal distributions of plants detected in *N. tenellus* gut contents, grouped into A) non-crop weeds, B) crops, and C) trees. The x-axis represents the collection date of leafhopper samples, while the y-axis lists the plant genera identified. The vertical dashed line corresponds to 21 June.

**Figure 1**

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**A map of a mountain

AI-generated content may be incorrect.**

**Figure 2**

**A graph of crops and crops

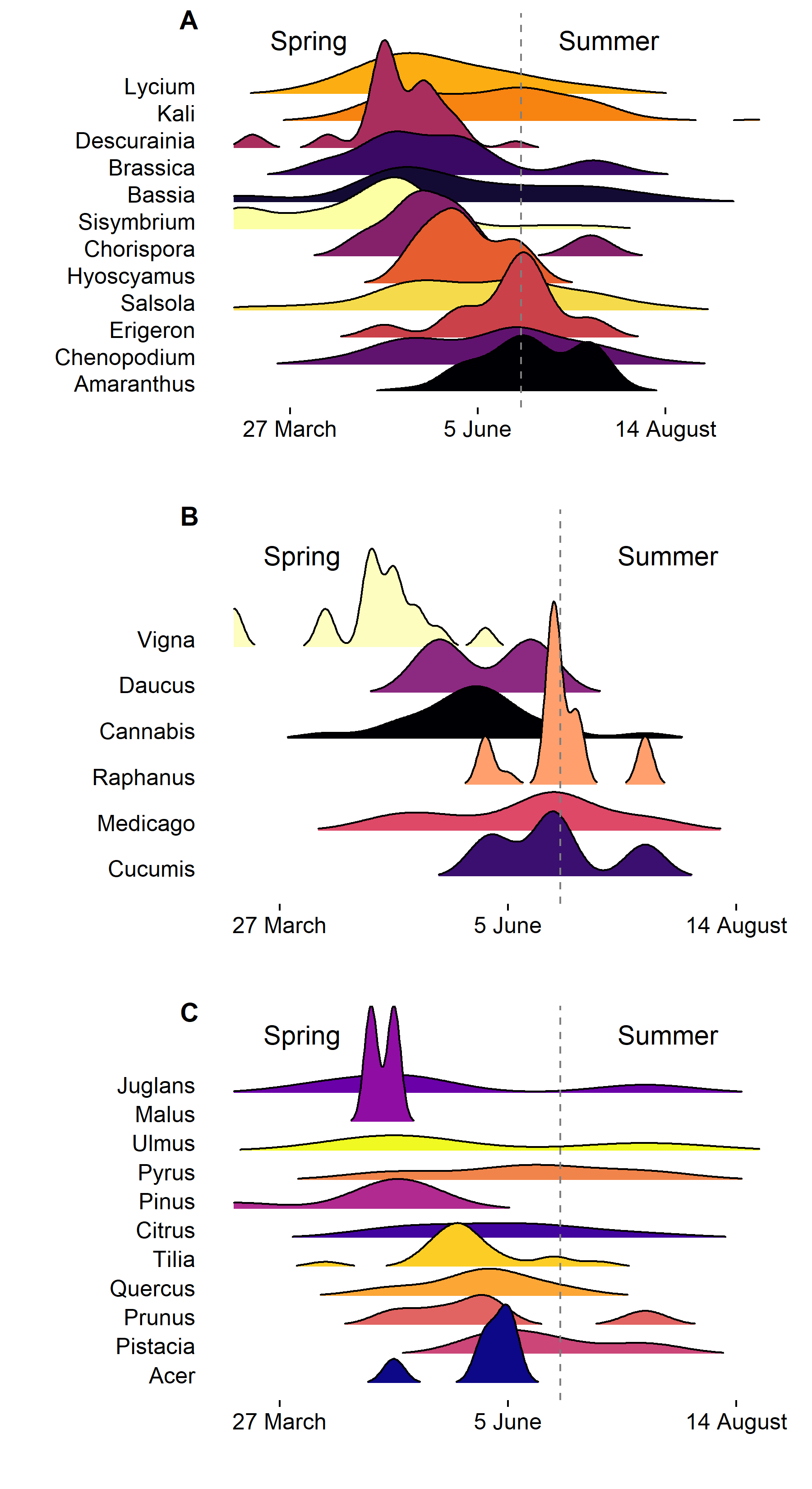
AI-generated content may be incorrect.**

**Figure 3**

**A diagram of a diagram of a planet

AI-generated content may be incorrect.**

**Figure 4**

****

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