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Research

Molecular ecology of host use and pathogen acquisition by an insect vector in potato crop fields

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Generalist insect vectors are notoriously difficult to manage due to their ability to use a range of host plants within and 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 has often been poorly linked with hosts related to pathogen transmission. Here, we integrate gut content analysis of vector insects with monitoring pathogen incidence to determine the role of various plant hosts in *Neoaliturus tenellus* (Baker) (beet leafhopper) movement and pathogen spread. We tested 226 beet leafhopper adults collected from 24 potato fields and nearby weeds over 3 growing seasons to assess host use and acquisition of beet curly top virus and '*Candidatus* Phytoplasma trifolii'. We observed seasonal variation in pathogen acquisition from host plants with *N. tenellus* acquiring pathogens from *Sisymbrium* spp. and *Brassica* spp. (wild mustards) in spring, whereas *Salsola/Kali* spp. (Russian thistle) and *Bassia* spp. (kochia) serve as the primary insect hosts during summer. Congruent with other recent research, we detected tree DNA in the guts of *N. tenellus* confirming tree probing or feeding, including *Tilia* spp. (linden), *Prunus* spp./*Pyrus* spp./*Citrus* spp (fruit), and *Tsuga* spp./*Pinus* spp. (pine). Our study refines our understanding of *N. tenellus* ecology and highlights the importance of host use patterns in predicting pathogen transmission, ultimately improving pathogen risk assessments.

Keywords: gut content analysis, beet curly top virus, purple top disease, landscape ecology, vector monitoring

Introduction

Many insect pests that are plant pathogen vectors are generalists that feed on diverse hosts across landscapes and seasons (Weintraub and Beanland 2006, Gutiérrez-López et al. 2020). Generalist vectors are difficult to manage because they move between crop and non-crop hosts (Nault and Ammar 1989, Heck 2018, Gutiérrez Illán et al. 2020). Areawide management is challenging, and large patches of unmanaged weedy hosts near commercial production areas can act as vector sources during crop seasons (Bennett 1971). The pathogens transmitted by these vectors also often infect many host species, although infectivity traits may not always align with vector host preferences (Thapa and Ghersi 2023). Understanding host use by vectors and pathogens is key for predicting vector and pathogen dynamics and aids in developing mitigation strategies.

Effective vector management requires innovative approaches to assess host use over space and time (Rafter and Walter 2020). One technique is molecular gut content analysis, which identifies plant DNA within insect digestive systems to elucidate trophic interactions (Cooper et al. 2019, 2022, Pitt et al. 2024). When molecular gut content analysis is used with structured insect monitoring, the technique can infer movement across landscapes across seasons (Cooper et al. 2019, Strausbaugh et al. 2024). Integrating gut content analysis with pathogen incidence data could clarify the relationships between host use and pathogen spread without observing direct feeding. However, few studies have assessed both vector gut content analysis and pathogen incidence. For example, one of the most notorious insect vectors in the western United States is the beet leafhopper, *Neoaliturus tenellus* (Baker; Hemiptera: Cicadellidae) (previously known as *Circulifer tenellus*), a species that feeds on over 300 host plant species (Strausbaugh et al. 2008). Yet, there are only 2 molecular gut content studies of this pest, and both focus on a single pathogen (Cooper et al. 2022, Strausbaugh et al. 2024).

The ecology of many insect vectors, such as *N. tenellus* are 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 are pathogen reservoirs, and pose the greatest risk to crops, are inferred without supporting data (Horton et al. 2018). For example, in the absence of data, it is often assumed that plant hosts used by a vector are also potential hosts for pathogens transmitted by that vector, or that plant hosts will be a

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predictable subset constrained by plant phylogeny (Perilla-Henao and Casteel 2016). Molecular tools can bridge this knowledge gap because the same insects can be tested for the pathogens they carry and their prior plant host use (Cooper et al. 2019, 2022, Pitt et al. 2024, Strausbaugh et al. 2024).

Here, we leveraged molecular gut content analysis to trace landscape-scale movements of *N. tenellus* in the Columbia River Basin region of Washington. As the sole vector of beet curly top virus (BCTV), '*Candidatus* Phytoplasma trifolii' (CPt) (also known as beet leafhopper-transmitted viresence agent), and *Spiroplasma citri* (*S. citri*), *N. tenellus* facilitates pathogen spread across seasons (Cooper et al. 2023). Monitoring suggests that wild Brassicaceae species serve as *N. tenellus* overwintering reservoirs, while Amaranthaceae species support populations during the growing season (Horton et al. 2018). However, it is still unknown which non-crop and crop hosts are primary pathogen reservoirs. Over 3 yr, we analyzed gut contents from 226 adult *N. tenellus* from 24 sites to assess seasonal host use and pathogen incidence to identify peak periods of *N. tenellus* activity and pathogen carriage. This study also highlights key non-crop host plants that maintain *N. tenellus* and pathogen populations that could support targeted weed management for inoculum reduction. Collectively, timely pesticide application and weed removal could reduce vector pressure and disease incidence in susceptible crops.

Materials and Methods

Study System

Leafhopper-transmitted pathogens threaten many crops in the western United States, including potatoes, hemp, and seed crops like sugar beet, carrot, coriander, and spinach (Soto and Gilbertson 2003, Munyaneza et al. 2006, Harveson 2015, Rondon and Murphy 2016, Giladi et al. 2020, Swisher Grimm et al. 2021). Gut adaptations of *N. tenellus* allow pathogens to traverse the stomach lining and colonize the salivary glands, facilitating transmission (Knowlton 1929, Suzuki et al. 2006, Chen and Gilbertson 2016, Koinuma et al. 2020, Frantz et al. 2023, Alkhatib et al. 2024). Further, *N. tenellus* lack strong host specificity and feed on many plants that do not support oviposition or development. Pathogen persistence thus depends on the lifecycle of *N. tenellus* and movement between weeds where acquisition occurs and hosts that support population increases, which include many crops (Thomas and Boll 1977, Meyerdirk and Hessein 1985, Meyerdirk and Moratorio 1987, Creamer 2020). This movement plays a role in BCTV and CPt transmission, yet how host use relates to pathogen prevalence is poorly understood. To characterize seasonal host use of *N. tenellus* and its role in spreading BCTV and CPt, we analyzed molecular gut contents using insect monitoring data from 24 sites.

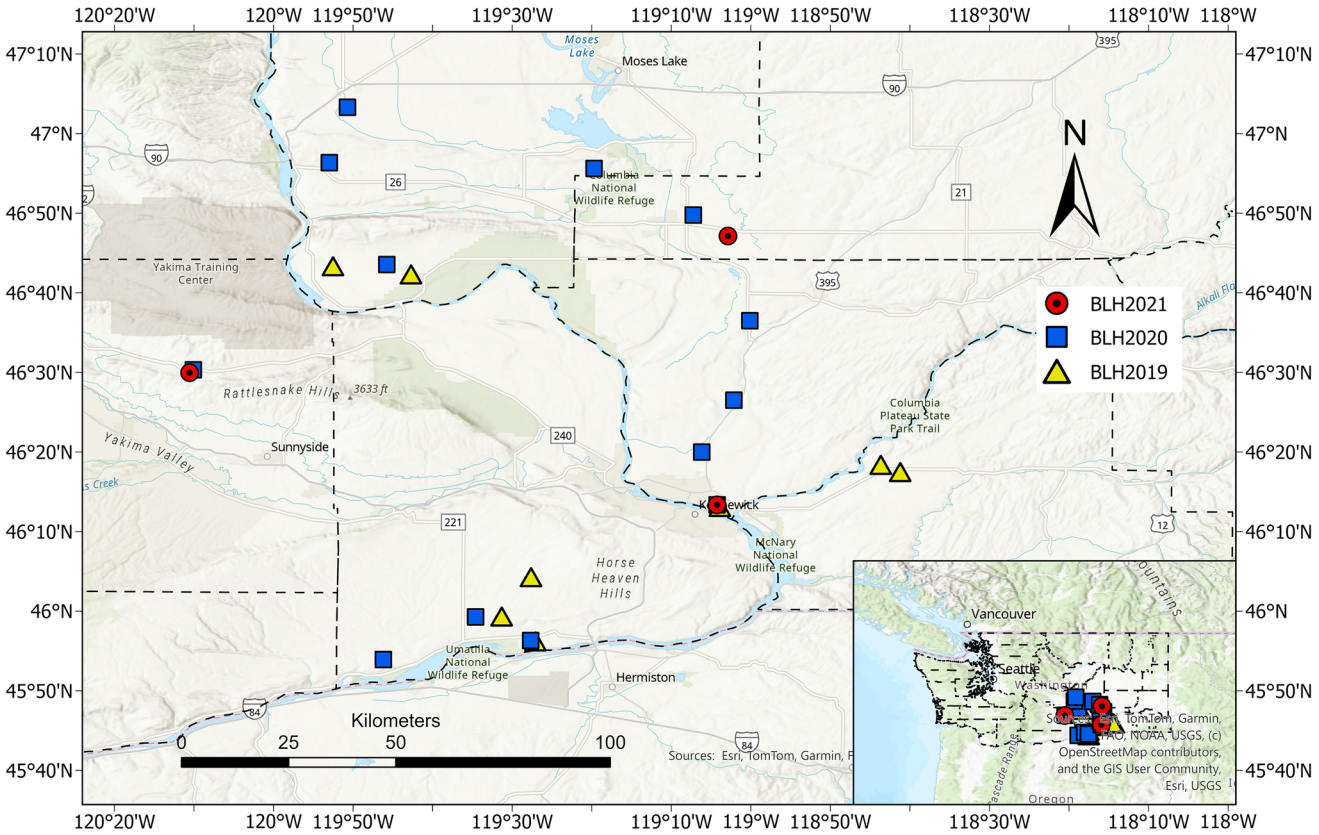

Fig. 1. Map. Collection sites for *Neoaliturus tenellus* by year.

Site Selection and Sampling

Sampling was conducted at sites across the Columbia River Basin during the growing seasons of 2019 and 2020, and spring 2021 (Fig. 1). Most sites were in Washington State University's potato pest monitoring network, a standardized 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). Sampling was conducted from early June through late August and again in late October in 2019, from late April through late August and again in early October in 2020, and from late March to early June in 2021. Our approach aimed to identify plant species that may serve as *N. tenellus* hosts at different times of the year. Our concurrent study tested plant samples for the presence of pathogens (Foutz et al. 2025). Linking vector gut content analysis with pathogen incidence may bring to light host–pathogen associations to improve predictive models for disease outbreaks (Cooper et al. 2019, Foutz et al. 2025).

We sampled every 2 wk in 2019 and 2020 and weekly in 2021 with a reversible leaf blower into organdy bags (26 cc gas handheld blower vacuum, Homelite Corporation, Charlotte, North Carolina). Edges of potato fields and potential weedy insect host stands within 100 m were sampled for 1 min. Live insects were transported on ice for a maximum of 8 h and then froze at −40 °C in the laboratory. Insects were identified and sexed under a stereo microscope using distinct morphological traits of tapered, bullet-body shape measuring 3 to 4 mm, with a pale green to tan coloration; high-definition images of the *N. tenellus* adults used have been published and describe these phenotypic traits (Foutz et al. 2025).

Of the 1,765 adult *N. tenellus* collected from 2019 to 2021, we used 226 individuals for gut content analysis with selection guided by year-specific research objectives. Each individual *N. tenellus* was sequenced using 2 different types of primer pairs. Around 100 primer pair combinations were evaluated per primer type, all of which consistently amplified both positive and negative controls, but the proportion of samples analyzed per year varied depending on primer performance. These *N. tenellus* samples were also tested to determine infection status, as described in the Pathogen Identification section.

In 2019, *N. tenellus* were selected for testing based on the plant species on which they were collected to confirm the plant was being fed upon and identify plants associated with pathogens. A total of 64 *N. tenellus* were subsampled based on collection from kochia, potato, Russian thistle, pigweed, and mustards. However, low pathogen incidence for CPt and BCTV limited our ability to identify reservoir hosts.

To address this constraint, the 2020 subsample was selected based on pathogen infection status rather than host plant. A total of 92 individuals were analyzed, including 23 infected with BCTV (one of which was co-infected with *S. citri*), 23 infected with CPt (one of which was co-infected with *S. citri*), 19 co-infected with both pathogens (one of which was tri-infected with *S. citri*), and 22 non-infected individuals. An additional 5 *N. tenellus* were infected solely with *S. citri*; however, due to the low incidence of this pathogen, *S. citri* was excluded from statistical analyses. This subsampling strategy allowed investigation of associations between infection status and host use.

Finally, 2021 sampling focused on addressing a key epidemiological hypothesis: That the first spring generation of migrating *N. tenellus* poses the greatest risk of introducing pathogens into crops. To explore this hypothesis, we selected 70 individuals collected during spring and early summer to characterize host use during the critical migration window.

This tiered sampling strategy ensured that individuals were selected to address the technical limitations of primer performance and the biological questions relevant to pathogen acquisition, overwintering ecology, and early-season transmission risk.

DNA Extraction

Adult *N. tenellus* were surface sterilized by immersing them individually in 70% ethanol for 5 s, sterile deionized water for 5 s, followed by a 60-s treatment in 1% bleach, and 2 final 5 s 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 assess DNA quality and concentration, samples were evaluated using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts). Extractions and subsequent testing included no-template controls using PCR-grade water to ensure that contamination had not occurred in any reagents. All extracted DNA samples were stored at −20 °C until further molecular procedures.

Pathogen Identification

Testing for the presence of 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 s, 65 °C for 10 s, and 72 °C for 10 s, then 20 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 10 s, 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, Massachusetts), 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 BCTV 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, Massachusetts), 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 ~520-bp PCR products under UV light on a 1% agarose gel stained with GelRed (Biotium, Fremont, California).

Samples from 2019 and 2020 were also tested for *Spiroplasma citri*, another plant pathogen transmitted by *N. tenellus*, using

conventional PCR with conditions identical for BCTV. 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 350-bp PCR products under UV light on a 1% agarose gel with GelRed staining. Only 8% of tested samples showed infection so this pathogen was left out of further analysis.

For molecular pathogen detection in this study, positive controls for all pathogen tests were created by combining aliquots of multiple confirmed positive *N. tenellus* samples. We used real-time PCR as presence/absence data were sufficient to address our 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). Thresholds were set automatically using the thermocycler program and varied slightly for each run but were typically around 37 cycles so that anything coming up higher was not counted as a positive.

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 2 common plant barcoding loci: the chloroplast trnF region and the nuclear 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 (Pacific Biosciences 2014), each sample was assigned a unique combination of asymmetric barcoded forward and reverse primers (Cooper et al. 2019, 2022). Reactions were performed in 50 µl volumes, using 40 µl Invitrogen Amplitaq Gold 360 PCR Master Mix at 62.5% (Invitrogen, Carlsbad, California), 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 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 45 s, with a final extension of 5 min at 72 °C. For trnF, cycling conditions were slightly adjusted: an initial denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, 58 °C (52 °C for some optimized reactions) for 30 s, and 72 °C for 45 s, and a 5-min 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).

Remaining PCR product volumes for pooling were adjusted based on band intensity, and cleaned using QIAquick PCR Purification Kits (Qiagen, Hilden, Germany). Each pooled set included no-template controls containing water and positive controls consisting of psyllid species collected from known host plants, each with a 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 h at the WSU Biotechnology and Bioanalysis Genomics Lab. Raw sequence data were processed using SMRT Link v6.0 to generate high-quality circular consensus sequences. 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 5 or fewer reads were excluded to minimize 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 if at least 6 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 were evaluated to explore seasonal trends in *N. tenellus* infection status and host plant use. The observational nature of this study precluded formal hypothesis testing, but we summarized our surveys to show the diversity of host plants, the variability in infection status, and the seasonal timing of host plant use by *N. tenellus*. We considered that variation in host 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 from which each *N. tenellus* was collected 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 and Willis 2003).

The effect of sampling date on host plant use and infection 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 and 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 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

Of the 226 *N. tenellus* tested across all 3 yr, 108 were pathogen-free, 39 harbored only BCTV, 42 harbored only CPt, and 28 carried both. In 2019 and 2020, we also tested for *S. citri* in 156 *N. tenellus*. Of this subset, 9 individuals tested positive. In 2019 only 1 *N. tenellus* was positive for *S. citri* with no other co-infections. Of the 8 *S. citri* positive individuals from 2020, 5 *N. tenellus* were positive for *S. citri* with no other co-infections, 1 was co-infected with CPt, 1 was co-infected with BCTV, and 1 tested positive for all 3 pathogens. The paucity of *S. citri* infections precluded its use in further testing in 2021 and analyses. Of the 226 insects tested, DNA barcoding detected 1,303 plant records across 107 genera, and 40 families (Table 1). We opted to partition these plants into 3 functional groups: crops, non-crops, and trees, and the subset of plants shown was selected on sample size. The majority of plants detected in *N. tenellus* guts were non-crop ($n = 893$, 68.5%), mainly *Bassia*, *Salsola*, *Sisymbrium*, *Amaranthus*, and *Kali* spp. (Fig. 2, Table 1). Crop genera were also found, primarily *Solanum*, *Raphanus*, *Cannabis*, *Medicago*, and *Vigna*; crops accounted for 26.3% ($n = 343$) of all plants found (Fig. 2, Table 1). We also detected multiple tree genera, although trees were comparatively rare in guts ($n = 67$, 5.1%) (Fig. 2, Table 1). Across all samples, *N. tenellus* individuals fed on a median of 5 plant species, 4 genera, and 3 families represented in gut contents, although individuals ranged from 1 to 20 species in gut contents. Consistent with the range observed, individual feeding breadth showed high variability at the plant species level (SD = 3.92).

Beet leafhopper infection status (BCTV, CPt, or both) varied independently across crop types with crops, non-crops, and trees all having similar infection proportions ($F_{6,132} = 0.89$, $P = 0.50$) (Fig. 2). *Spiroplasma citri* was excluded due to insufficient sample size for meaningful statistical comparison. Neither pathogen infection nor site location shaped dietary diversity, as plant community composition in *N. tenellus* guts was not influenced by CPt infection status ($F_{1, 210} = 1.44$, $P = 0.30$) or region ($F_{4, 210} = 1.38$, $P = 0.38$). In contrast, BCTV infection status had a significant effect on plant composition found in leafhopper guts ($F_{1, 210} = 3.62$, $P = 0.01$). See Table 2 for a tabular version of these results. This effect was not due to differences in within-group variability, as confirmed by permutation tests for homogeneity of multivariate dispersions ($F_{1, 224} = 2.39$, $P = 0.13$, permutations = 999).

Despite the significance of the BCTV infection term, this term explained only 1.0% of the variation in gut content composition (Table 2). In comparison, our constrained ordination showed that the first unconstrained axis (MDS1) captured 24.1% of residual variation (Fig. 3). Moreover, in constrained ordination, plant species vectors aligned primarily along the unconstrained axis, orthogonal to the constrained BCTV axis. This evidence suggests that while PERMANOVA detected subtle differences in BCTV infection status associated with certain plant species, the majority of variation in plant community composition is not aligned with BCTV infection status.

Plant community composition in *N. tenellus* guts varied significantly across sampling dates ($F_{5, 210} = 10.29$, $P < 0.01$; Fig. 3), though this effect was collinear with collection year. Variation in gut plant community over time was further supported by Kernal density estimation. Non-crop plants were more commonly detected earlier in the season (Fig. 4A), while crop plants appeared slightly later (Fig. 4B), with some exceptions and overlap. However, density plots with large magnitudes and multiple sharp peaks represent plants detected in only a few individuals, making true seasonal trends in plant consumption somewhat uncertain (Fig. 4).

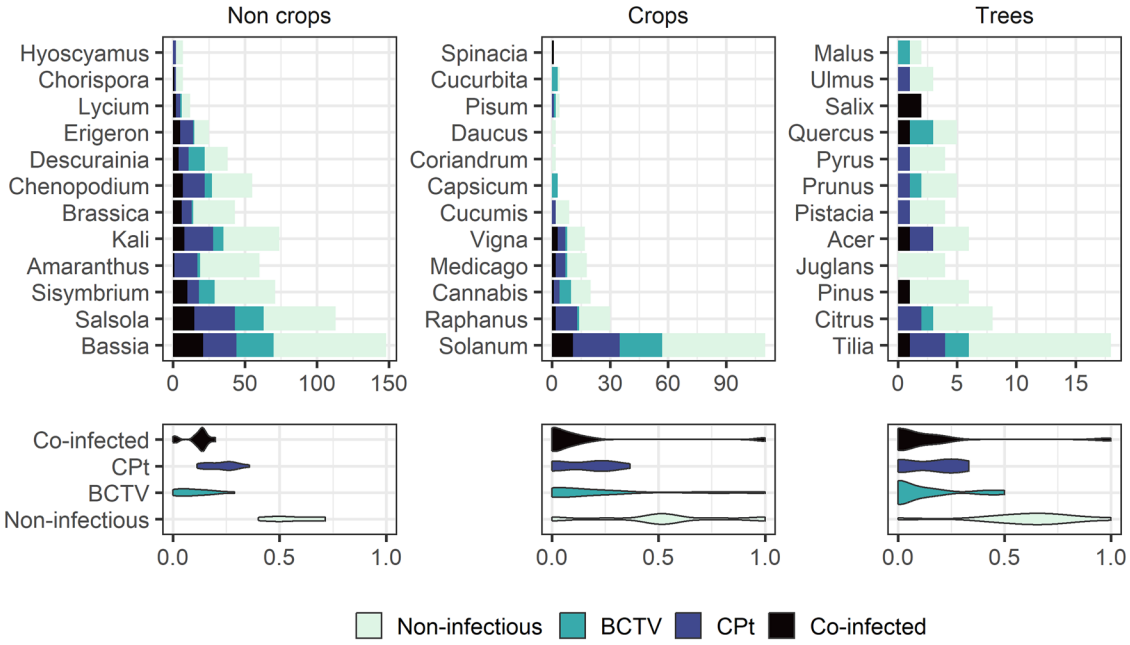

Fig. 2. *Neoaliturus tenellus* gut content analysis. Counts of plant genera in individual *N. tenellus* gut contents, categorized into 3 groups: non-crops (or weeds), crops, and trees. The *x* axis represents the count of leafhoppers in which each plant type was detected, while the *y* axis lists the plant genera identified. The lower panel shows the distribution of infection status across the 3 plant groups. Co-infection refers to the presence of both CPt and BCTV.

Table 1. List of plants found in *Neoaliturus tenellus* guts and their absolute and proportional abundances

Family	Genus	Detections	Percent
Solanaceae	*Solanum*	232	17.83
Amaranthaceae	*Bassia*	148	11.38
Amaranthaceae	*Salsola*	113	8.69
Amaranthaceae	*Kali*	74	5.69
Brassicaceae	*Sisymbrium*	71	5.46
Amaranthaceae	*Amaranthus*	60	4.61
Asteraceae	*Lactuca*	56	4.3
Amaranthaceae	*Chenopodium*	55	4.23
Brassicaceae	*Brassica*	43	3.31
Brassicaceae	*Descurainia*	38	2.92
Brassicaceae	*Raphanus*	30	2.31
Asteraceae	*Erigeron*	25	1.92
Poaceae	*Bromus*	25	1.92
Cannabaceae	*Cannabis*	20	1.54
Fabaceae	*Medicago*	18	1.38
Malvaceae	*Tilia*	18	1.38
Fabaceae	*Vigna*	17	1.31
Geraniaceae	*Erodium*	14	1.08
Solanaceae	*Lycium*	12	0.92
Convolvulaceae	*Convolvulus*	10	0.77
Cucurbitaceae	*Cucumis*	9	0.69
Poaceae	*Hordeum*	9	0.69
Rutaceae	*Citrus*	8	0.61
Brassicaceae	*Chorispora*	7	0.54
Solanaceae	*Hyoscyamus*	7	0.54
Pinaceae	*Pinus*	6	0.46
Sapindaceae	*Acer*	6	0.46
Brassicaceae	*Pseudofortuynia*	5	0.38
Fagaceae	*Quercus*	5	0.38
Rosaceae	*Prunus*	5	0.38
Amaryllidaceae	*Allium*	4	0.31
Anacardiaceae	*Pistacia*	4	0.31
Asteraceae	*Artemisia*	4	0.31
Cucurbitaceae	*Cucurbita*	4	0.31
Fabaceae	*Pisum*	4	0.31
Fabaceae	*Robinia*	4	0.31
Juglandaceae	*Juglans*	4	0.31
Oleaceae	*Fraxinus*	4	0.31
Poaceae	*Lolium*	4	0.31
Polygonaceae	*Fagopyrum*	4	0.31
Rosaceae	*Pyrus*	4	0.31
Solanaceae	*Physalis*	4	0.31
Asteraceae	*Centaurea*	3	0.23
Asteraceae	*Solidago*	3	0.23
Asteraceae	*Sonchus*	3	0.23
Asteraceae	*Tragopogon*	3	0.23
Boraginaceae	*Amsinckia*	3	0.23
Fabaceae	*Glycine*	3	0.23
Malvaceae	*Malva*	3	0.23
Poaceae	*Cenchrus*	3	0.23
Smilacaceae	*Smilax*	3	0.23
Solanaceae	*Capsicum*	3	0.23
Ulmaceae	*Ulmus*	3	0.23
Zygophyllaceae	*Tribulus*	3	0.23
Apiaceae	*Coriandrum*	2	0.15
Apiaceae	*Daucus*	2	0.15
Asteraceae	*Achillea*	2	0.15
Asteraceae	*Corethrogyne*	2	0.15
Asteraceae	*Helianthus*	2	0.15
Basellaceae	*Basella*	2	0.15
Convolvulaceae	*Ipomoea*	2	0.15

(Continued)

Table 1. Continued.

Family	Genus	Detections	Percent
Fabaceae	*Vicia*	2	0.15
Oleaceae	*Ligustrum*	2	0.15
Oleaceae	*Olea*	2	0.15
Poaceae	*Festuca*	2	0.15
Poaceae	*Poa*	2	0.15
Poaceae	*Secale*	2	0.15
Rosaceae	*Malus*	2	0.15
Rosaceae	*Photinia*	2	0.15
Rosaceae	*Rubus*	2	0.15
Salicaceae	*Salix*	2	0.15
Solanaceae	*Nicotiana*	2	0.15
Amaranthaceae	*Beta*	1	0.08
Amaranthaceae	*Spinacia*	1	0.08
Apocynaceae	*Leptadenia*	1	0.08
Asteraceae	*Ambrosia*	1	0.08
Asteraceae	*Carthamus*	1	0.08
Asteraceae	*Echinacea*	1	0.08
Betulaceae	*Alnus*	1	0.08
Betulaceae	*Betula*	1	0.08
Boraginaceae	*Phacelia*	1	0.08
Cucurbitaceae	*Citrullus*	1	0.08
Fabaceae	*Lotus*	1	0.08
Fabaceae	*Maackia*	1	0.08
Fabaceae	*Phaseolus*	1	0.08
Fabaceae	*Styphnolobium*	1	0.08
Lamiaceae	*Mentha*	1	0.08
Lauraceae	*Lindera*	1	0.08
Lauraceae	*Phoebe*	1	0.08
Linaceae	*Linum*	1	0.08
Lythraceae	*Lagerstroemia*	1	0.08
Morceae	*Morus*	1	0.08
Pinaceae	*Abies*	1	0.08
Plantaginaceae	*Plantago*	1	0.08
Platanaceae	*Platanus*	1	0.08
Poaceae	*Aegilops*	1	0.08
Poaceae	*Elymus*	1	0.08
Poaceae	*Pseudoroegneria*	1	0.08
Poaceae	*Setaria*	1	0.08
Poaceae	*Sorghum*	1	0.08
Poaceae	*Triticum*	1	0.08
Polygonaceae	*Polygonum*	1	0.08
Portulacaceae	*Portulaca*	1	0.08
Sapindaceae	*Dimocarpus*	1	0.08
Solanaceae	*Scopolia*	1	0.08
Theaceae	*Camellia*	1	0.08
Vitaceae	*Vitis*	1	0.08

Discussion

Identifying seasonal host plants of *N. tenellus* can aid in understanding vector movement across landscapes and inform targeted management strategies for vector-borne pathogens. Gut contents of *N. tenellus* were primarily composed of non-crop plants, with non-crop abundance typically 1 order of magnitude greater than crop or tree abundance. It is well established that *N. tenellus* feed on a broad range of plants, with over 40 commonly used non-crop host plants that vary across different breeding regions (Douglass and Hallock 1957). Hundreds of dicotyledonous species are fed upon by *N. tenellus* and are susceptible to infection by BCTV and CPt, but *N. tenellus* also feed on monocots, which are unsuitable for these pathogens despite being part of the insect's diet (Supplementary Fig. S1) (Chen and Gilbertson 2016, Strausbaugh et al. 2024). In our study, we detected relatively few monocot taxa, primarily *Lolium, Bromus, Setaria, Poa*, and *Triticum* spp.

Some individual *N. tenellus* contained DNA up to 20 different plant species, suggesting frequent movement among hosts. However, plant species in the guts were not associated with infection status, which may represent broad generalism, uniform pathogen dispersion, or low pathogen presence in some genera. A previous study of *N. tenellus* feeding showed that non-viruliferous leafhoppers preferentially probed barley (*Hordeum vulgare*, a non-host for BCTV) over tomato (*Solanum lycopersicum*, a BCTV host), whereas viruliferous

insects showed no preference (Lee et al. 2022a). Behavioral changes from viral infection may be relevant to our observation that viruliferous individuals had fewer plant species detected in guts (Han et al. 2024), although seasonal sampling differences complicate interpretation. Another plausible explanation is that hypersensitivity in molecular tools may have detected trace amounts of ubiquitous plants. For example, many of the common weeds (*Kali*, *Bassia*, *Salsola*, *Sisymbrium*) appeared in 90% of the *N. tenellus* guts assayed. Shifts in dietary composition with BCTV or CPt infection may still exist and could be a potential driver of vector behavior and host selection.

By sampling from early spring to fall, our surveys capture the seasonal variation in host use. This lends support for the spring migration hypothesis that *N. tenellus* may migrate from early emerging weeds to crops throughout the summer. In early spring, gut contents were marked by peak abundance of non-crop plants such as *Bassia*, *Descurainia*, *Sisymbrium*, and *Lycium*. Most crops (*Solanum*, *Cannabis*, *Raphanus*, *Medicago*, and *Cucumis*) were found in peak abundance in gut contents in late spring to early summer. Some plants were found in *N. tenellus* during the season at near uniform distributions (*Salsola* and *Kali*). These ubiquitous all-season plants may represent reservoirs for reinfection throughout the season, in line with prior work (Douglass and Hallock 1957, Munyaneza et al. 2006, Foutz et al. 2025). Further evidence of host plant species turnover was found in our multivariate results, with sampling date representing most of the variation in species composition that could be explained by our predictors.

Our results suggest that wild mustards, particularly *Sisymbrium* and *Descurainia* species, are common spring hosts for *N. tenellus*, with peak mustard feeding coinciding with the early-season emergence of leafhopper nymphs (Foutz et al. 2025). These mustard hosts support *N. tenellus* development and early pathogen acquisition due to their ability to overwinter, cold hardiness, and tendency to germinate early (Cici and Van Acker 2011, Foutz et al. 2025). We also found common tree species including *Tilia*, *Pyrus*, *Prunus*, and conifers such as *Pinus* and *Tsuga*, suggesting that trees may be probed or fed upon by *N. tenellus*. More research is needed to test trees for *N. tenellus*-vectored pathogens to determine their susceptibility. Previous research using different DNA barcoding primers has also identified unexpected plants in *N. tenellus* diets, including coniferous trees such as *Pinus* spp., particularly during early summer dispersal events (Cooper et al. 2022, Strausbaugh et al. 2024). These findings further support that *N. tenellus* may feed on a broader range of plant taxa—including trees—than previously recognized. These behaviors have important implications for understanding their movement ecology and the epidemiology of the pathogens they transmit (Strausbaugh et al. 2024).

The timing of tree species host use appears to coincide with spring bolting of mustards. While not tested directly here, this

Table 2. Summary statistics from a PERMANOVA fitting a matrix of plant community composition in *N. tenellus* guts as a function of sample date (a spline term with df = 5), BCTV infection status, collection year, CPt infection status, collection region, and the BCTV × year interaction

Factor	df	∑(squares)	R^2	F	P
Sample data (spline)	5	10.0	0.16	10.3	<0.0001
BCTV	1	0.70	0.01	3.62	0.01
Year	2	8.16	0.13	21.0	<0.0001
CPt	1	0.28	0.00	1.44	0.30
Region	4	1.07	0.02	1.38	0.38
BCTV × Year	2	0.63	0.01	1.63	0.23
Residual	211	41.0	0.66	-	-
Total	226	61.8	1.00	-	-

The host plant from which each *N. tenellus* collected was used to stratify the PERMANOVA with a random intercept.

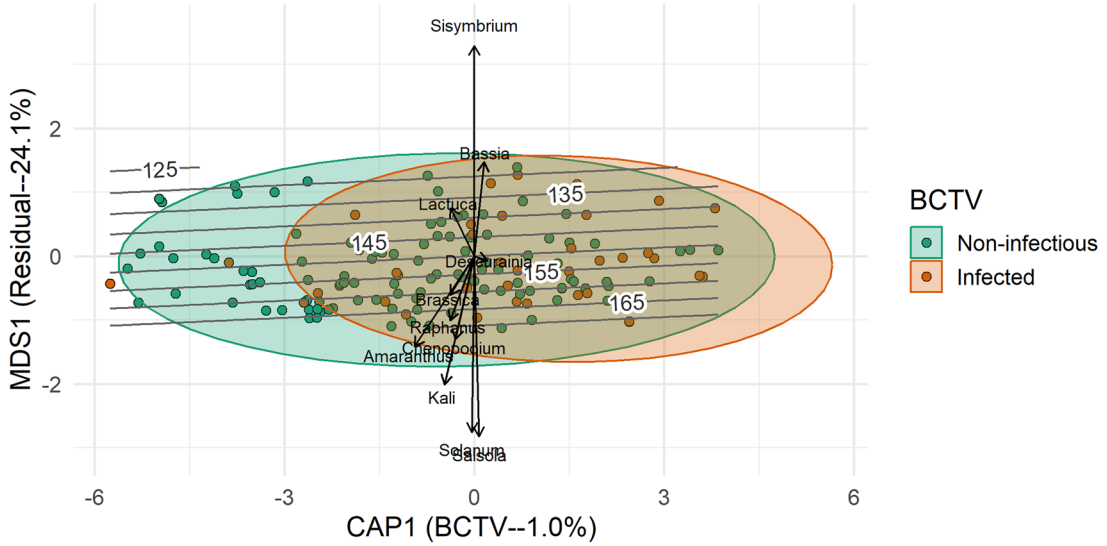

Fig. 3. Canonical analysis of principal coordinates for gut contents. CAP1 represents variation in gut content plant community composition constrained to show separation by BCTV infection status. MDS1 represents the first major axis of residual (unconstrained) variation in community composition. Points 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.

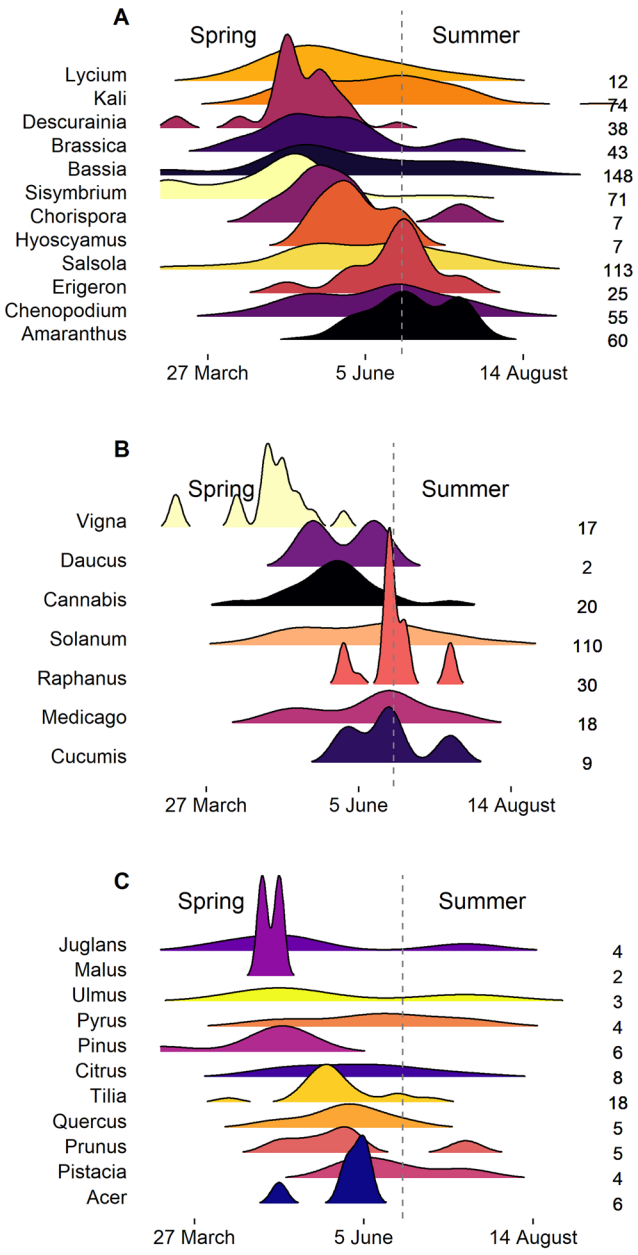

Fig. 4. Seasonality of *Neoaliturus tenellus* gut content analysis. Genus-level seasonal distributions of plants detected in *N. tenellus* gut contents, grouped into (A) non-crops, (B) crops, and (C) trees. The *x* axis represents the collection date of leafhopper samples, while the *y* axis lists the plant genera identified. The vertical dashed line corresponds to 21 June. The magnitude of the ridges represent density at each timepoint proportional to the sample size of each genus (numbers to the right of ridges). Genera with small sample sizes will have larger magnitudes and greater uncertainty of the true seasonal distribution.

phenology timing may serve as an important biomarker for tree crop growers, as trees may 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 (Foutz et al. 2025), while Russian thistle (*Salsola* spp.) was the most frequent genus overall. Seasonal host shifts appear driven by temperature and host availability (Lee et al. 2022b), 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). While plants like *Salsola* were commonly detected 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 pathogen 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.

While these findings offer important insights, several limitations must be acknowledged. Sampling was not uniform across years, and 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. Additionally, gut content analysis may have potential limitation due to primer specificity of the DNA barcoding primers used, which may bias results toward certain genera. To address this, we used 2 primer types, ITS2 for broad taxonomic identification and trnF for targeting specific weed species. Additionally, results were validated at the genus level to account for potential species-level misassignments caused by digestion or sequencing error. Despite these precautions, some low-abundance hosts may be underrepresented, and additional analysis with more leafhoppers could further confirm patterns observed here.

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 genera, seasonal trends, and differences in pathogen–vector interactions, our findings can be incorporated into broader integrated pest management frameworks to offer opportunities to reduce pesticide use, lower grower costs, and promote long-term agricultural sustainability. Growers currently rely on intensive insecticide applications, costing around $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, potentially reducing unnecessary pesticide applications. 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, which provides precision pest management recommendations

and has already saved potato farmers $9 million annually (Wohleb et al. 2021, WSU Decision Aid System 2025). Models that link vector movement with host use patterns across seasons may offer new opportunities for more precise pesticide application timing and location.

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Author Contributions

Camille Wagstaff (Conceptualization [lead], Data curation [lead], Formal analysis [equal], Methodology [equal], Writing—original draft [supporting], Writing—review & editing [equal]), Jillian Foutz (Data curation [equal]), Rodney Cooper (Methodology [lead], Writing—review & editing [equal]), Riley Anderson (Formal analysis [lead], Writing—review & editing [equal]), and David Crowder (Funding acquisition [lead], Investigation [lead], Project administration [lead], Resources [lead], Supervision [lead], Writing—review & editing [lead])

Supplementary Material

Supplementary material is available at *Annals of the Entomological Society of America* online.

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Conflicts of Interest

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the Washington State University (WSU) or the U.S. Department of Agriculture (USDA). WSU and USDA are equal opportunity providers and employers.

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